THE GROWTH OF THE ODONTOBLASTS OF THE INCISOR TOOTH AS A CRITERION OF THE VITAMIN C INTAKE OF THE GUINEA PIG[1]

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FIVE FIGURES

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After describing briefly 2 physical methods, 21 chemical methods, and 1 biochemical method, Rosenberg ('45) stated "Although the chemical, and to a small extent also, physical methods are replacing more and more the biological determinations of vitamin C, the biological tests maintain their place as the ultimate and most correct method of determining vitamin C." The problem of the assay of this vitamin was of particular concern to the Canadian Government during the war years because of the difficulty of providing natural sources of vitamin C to the armed forces for a considerable portion of the year. Inasmuch as different chemical procedures frequently gave different results as to the potency of a food in which the armed forces were interested, this laboratory was requested in 1942 to undertake the establishment of a vitamin C bioassay which might be used as a check against chemical procedures.

THE BASAL DIET

Most biological assays for vitamins depend ultimately on the normal development of the experimental animal, either as

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a whole, as measured by growth; or of some tissue which can be observed separately and on which the vitamin appears to have specific effects. Since the development of the tissues of the body may be limited through diet inadequacies, it is obviously essential that any diet, to be fully satisfactory as the basis of a bioassay, must be nutritionally complete with the exception of the vitamin to be studied.

Insofar as we are aware, the nutritional requirements of the guinea pig are not as yet completely known — in the sense at least that it has not yet been possible to provide either a purified diet or one of natural foods which is fully successful in maintaining normal growth and reproduction, unless some fresh herbage is also fed. Several of the diets, fortified with ascorbic acid, which have been reported for the bioassay of vitamin C have proved in this laboratory to be entirely inadequate to support a successful pregnancy or to promote normal growth of young pigs to maturity.

We were faced at once, therefore, with the necessity of developing a basal diet for the assay. Suitable criteria of nutritional adequacy offered some problems, but due to the fact that the young guinea pig has a particularly long intrauterine life, and that it is physiologically much more mature at birth than most other small animals, it was believed that dietary deficiencies might be manifest by reproductive failure or by sub-normal performance of breeding females confined to such rations. It was decided, therefore, to feed to pregnant guinea pigs, different basal diets suitable with respect to such factors as palatability, physical nature and freedom from vitamin C, and to note their ability to complete normal reproduction. Comparable females, fed the same diets plus green feed, were used as controls. All test basal diets were supplemented with ascorbic acid as the sole source of vitamin C.

In addition to the pregnancy tests, the more promising of the mixtures were subjected to growth-tests with groups of young animals fed against check groups receiving the same rations plus green feed.

As a result of these studies a basal diet has been devised for the vitamin C bioassay. It is called the Macdonald Guinea Pig Basal Diet no. 5, and has the following percentage composition: ground oats 15, ground wheat 13,[2] ground, dried beet-pulp 25, linseed oilmeal 12.5, skimmilk powder 15, fishmeal 5, brewer's dried yeast 10, bone char 4, and salt (iodized) 0.5.

For feeding, the mixture is pressed into pellets of about one-eighth inch diameter. We have found that while guinea pigs will scratch a meal mixture out of their feeders, they will eat the same mixture without waste when it is offered in small pellet form. In addition to the dry basal mixture, the pigs were supplied directly with: vitamins A and D as a feeding fish oil; vitamin E as alpha tocopherol; and vitamin C as ascorbic acid. It may be noted that wheat germ to the extent of 10% of the basal mixture did not prevent muscle degeneration and hemorrhage which were entirely corrected by daily allowances of 3 mg of alpha tocopherol.

In our experience, when this vitamin-supplemented ration is fed ad libitum along with either fresh or with dried, long-stored grass clippings, reproduction (80% successful pregnancies) and the growth of young are normal; whereas if the roughage is omitted, only about 66% of pregnancies are successful and there is some, though not a marked slowing of growth of the young to maturity. It may be noted that the dried herbage clippings used were not anti-scorbutic. Because of the evident craving for edible roughage, it is interesting to speculate as to whether or not some of the failure which has been reported in the preparation of purified diets for guinea pigs is not related to the need of this species for roughage material to maintain normal caecum function, comparable to the case of the rumen with ruminants.

From our observations of several hundred pregnant guinea pigs and of their progeny, we have now constructed what may

[2] Five pounds of this component may be replaced, if desired by an equal weight of molasses for greater ease in pelleting.

be called a "normal standard" for the behavior of guinea pigs on this diet regime. It may be summarized as in table 1.

Although it is evident from these tests that the diet which we are using without roughage for the odontoblast assay is not entirely complete nutritionally when judged by the severe criterion of adequacy for reproduction, yet young pigs carried on bioassay, on this diet, show no abnormalities at post-mortem examination at the end of the 42-day assay period provided the ascorbic acid has been supplied in amounts of 2 mg per pig per day or greater.

TABLE 1

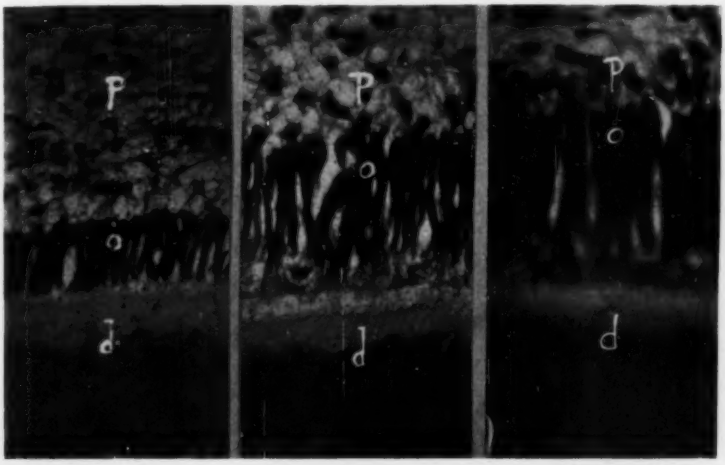
Typical average performance of guinea pigs on vitamins A, C, D and E supplemented Macdonald guinea pig diet no. 5.

RESPONSE WITH REGARD TO:	WITHOUT ROUGHAGE	WITH FRESH OR DRIED ROUGHAGE
Successful pregnancies	67%	80%
Hemorrhage and resorption of fetus	20%	20%
Abortion	13%	0%
Number of pigs per litter:		
total	3.7	3.9
born alive	3.0	3.5
weaned	3.0	3.5
Birth weight of pigs born alive	100 gm	100 gm
Weight at 21 days (weaning time)	260 gm	275 gm
Gains per wk. from weaning time to 500 gm (females only)	30 gm	43 gm

The bioassay procedure

Numerous workers have described the radical changes which occur in the scorbutic guinea pig tooth and these changes have been used in subjective assay methods as an index of the vitamin C intake of young guinea pigs. All of these methods suffer from an inherent lack of precision. In 1940, an objective method of assay was proposed by Boyle, Bessey and Howe based on their finding that the width of the dentine layer of the incisor tooth varied with the vitamin C intake. This method

appeared to avoid the difficulty of assessing the degree of tooth damage involved in earlier tooth assay methods. However, in examining the teeth of pigs on varying intakes of ascorbic acid, we were impressed with the clear-cut and easily measured differences in the length of the odontoblast cells (see fig. 1).



0.5 mg 1.0 mg 2.0 mg

Fig. 1 Photomicrographs (× 440) showing development of the odontoblast cells in guinea pig incisor teeth resulting from the daily intake of 0.5, 1.0, and 2.0 mg of ascorbic acid, respectively. P, o, and d indicate pulp, odontoblasts and dentine, respectively.

In the mildly scorbutic guinea pigs the odontoblast cells at the formative end of the incisor tooth appear practically normal, while further incisally they become shorter and irregular in position (see fig. 2).

In the normal guinea pig, the odontoblasts, differentiating at the formative end of the tooth, increase in length until they reach maturity. At the senile end of the tooth, the odontoblasts have degenerated and are embedded in calcific scar tissue, or secondary dentine. Between these 2 well-defined

regions lies a row of tall columnar mature odontoblasts. Cell measurements must be taken in this area (figs. 3, 4 and 5).

These changes can be seen in longitudinal sections cut to expose the full length of the pulp cavity of the incisor tooth

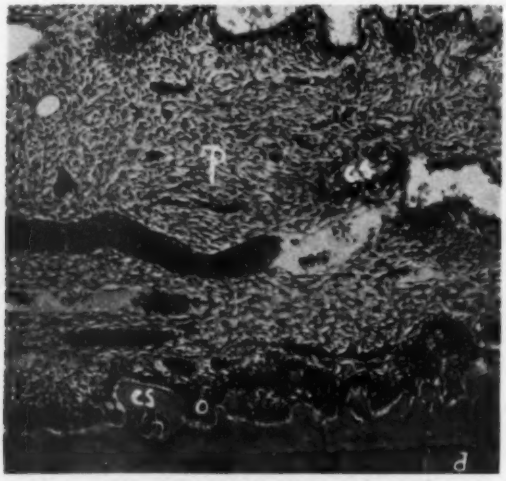


Fig. 2 Photomicrograph (× 100) showing irregular odontoblast row in a scorbutic tooth. (P) indicates disorganized pulp, (es) calcific scar tissue, (o) degenerating odontoblasts, and (d) dentine, respectively.

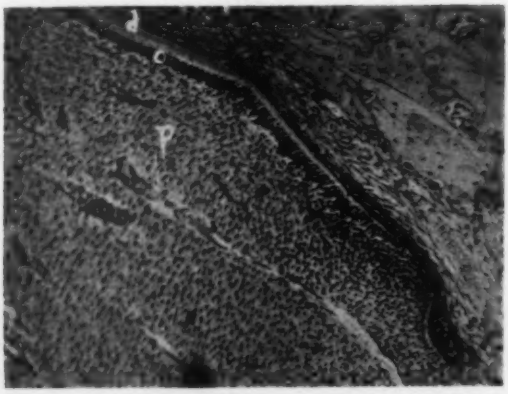


Fig. 3 Formative end of incisor tooth showing embryonic odontoblasts.

and are easily measured with a microscope fitted with a micrometer eye piece.

The length to which these cells develop in pigs of 250 to 400 gm weight, is apparently limited by the level of vitamin C intake, and ranges from about 30 microns with intakes of 0.5 mg of ascorbic acid daily, to a maximum of about 70 microns with intakes of 2 mg or over. Within this range of

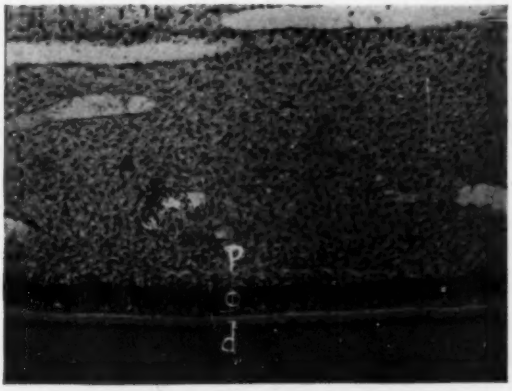


Fig. 4 Mature odontoblast cells in area of reading.

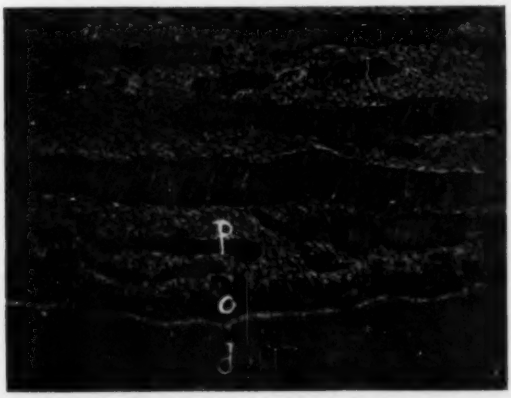


Fig. 5 Incisal end of incisor tooth showing senile odontoblasts. (P) indicates, pulp, (o) odontoblasts, and (d) dentine, respectively.

intake, the odontoblast length bears a logarithmic relation to the vitamin C fed. With intakes of less than 0.5 mg the cells are so disorganized that accurate readings are difficult, and in addition, frank scurvy is present in many individual animals which, in our opinion, renders them unacceptable for assay purposes.

Allotment considerations

We have now conducted tests involving some 650 pigs, aimed at establishing an assay procedure having the obviously desirable characteristics of precision, simplicity and reliability.

In this connection it may be stated that when raised to assay age on our vitamin supplemented basal diet, the absence or use of green feed is apparently immaterial. Further, there are no differences between the sexes in odontoblast cell development and the animals may be penned alone or in groups during the assay. Somewhat more regular curves have been obtained in assays of 35 and 42 days than on shorter periods, and selection of pigs for assay on the basis of age (28 days) has been preferable to the use of an attained live weight of 300 ($\pm$ 5) gm.

Limits of assay range

The response to ascorbic acid of pigs carried under assay conditions has been measured at daily intakes of 0.25, 0.50, 1.0, 2.0, 4.0, and 8 mg. In our experience, pigs carried for over 28 days on intakes of 0.25 mg of ascorbic acid have shown frank scurvy including capillary hemorrhage in the hind legs. No cases of clinical scurvy have been seen on intakes of 0.5 mg per day.

At the upper end of the range we have not found satisfactory increased response with intakes of 4.0 or 8.0 mg over that shown for 2.0 mg. We have not thus far investigated values intermediate between 0.25 and 0.5 mg, or between 2.0 and 4.0 mg of ascorbic acid.

Precision of the assay

In all tests, in addition to the odontoblast readings, the gains of the animals have been recorded and a gain response curve determined. When male animals only are used, the mean estimated potency of the carrier was frequently approximately the same as determined from the odontoblast readings. The response of the females, however, was usually unsatisfactory because of much slower gains.

The relative precision of the 2 methods (gain vs. odontoblast) is striking. The precision of an assay of this type may be expressed as the ratio of the standard error of the mean difference between 2 levels of vitamin intake and the slope of the regression connecting them,

$$pr = \frac{\sigma}{b} \times \sqrt{\frac{2}{n}} \qquad (1)$$

where n is the number in the group, σ the standard deviation of the assay, and pr the precision.

One measure of the relative precision may be indicated by the number of animals needed per group to give the same values for pr. For this purpose we may write the above formula in the form of

$$n = \frac{2}{pr^2} \times \frac{\sigma^2}{b^2} \qquad (2)$$

The number of animals needed per group (i.e., test level group) where growth is the criterion to give a precision *(pr)* equal to that attained with the number actually used for the odontoblast readings may then be calculated by using in formula (2) the values of pr^2 found for the odontoblast readings and those of σ^2 and b^2 found for the growth values. The n thus found will be compared to the n used in the odontoblast calculations (see column "nc" of table 2).

Another way of indicating relative precision involves a comparison of the precision indexes of the 2 methods. This is shown in the last column of table 2.

The relevant data for 7 separate assays are given in table 2. In our experience the odontoblast method has consistently given an appreciable increase in precision over the growth method of estimating vitamin C intake.

TABLE 2

Relative precision of growth vs. odontoblasts as criteria of vitamin C intake for guinea pigs.

SUBSTANCE ASSAYED	ODONTOBLAST METHOD				GROWTH METHOD				nc [1]	RELATIVE PRECISION
	a	b	n	pr	a'	b'	n'	pr'		
Aqueous soln. ascorbic	2.8	32	10	.039	49.3	247	10	.089	53	.44 [2]
Fresh orange juice	3.5	21	10	.074	56.5	61	10	.415	314	.18
Conc. orange juice	5.4	59	6	.053	26.5	135	6	.113	28	.47
Aqueous soln. ascorbic	3.2	21	10	.070	57.6	105	10	.244	122	.29
Fresh orange juice	3.2	18	10	.082	57.6	122	10	.211	66	.39
Synthetic orange juice	3.2	22	10	.065	57.6	94	10	.273	177	.24
Apple juice	3.2	33	10	.044	57.6	192	10	.134	95	.33

[1] nc in growth assay to give pr equal to odontoblast assay.

[2] *i.e.*, Growth assay has 44% the precision of the odontoblast assay.

Details of assay procedure

The details of the procedure now used in this laboratory are as follows:

Young guinea pigs bred in our own colony and raised from mothers maintained on the basal diet, above described, supplemented with vitamins A, C, D and E plus either hay or greenfeed as available, are at 28 ($\pm$ 3) days of age, allotted at random to individual cages and allowed free access to the pelleted basal diet and water. Vitamins A, D and E in corn oil are administered weekly, by means of a calibrated hypodermic syringe, with needle removed, in amounts to provide the equivalent of 425 I.U. of vitamin A, 48 I.U. of vitamin D and 3 mg of alpha tocopherol daily.

Both the unknown and the control source of vitamin C are fed at each of at least 3 levels of intake, spaced at equal log intervals. We have continued the restriction in allotment that there shall be equal numbers of each sex on each dose level,

and for convenience, have continued the use of individual penning. (Normally not less than 10 animals per dose are used.)

The feeding period for all pigs is 42 days. Since individual penning is employed, different pigs can start and hence complete a trial at different times. This is advantageous not only in the problem of obtaining the needed numbers of animals for assay at any given time, but especially in avoiding the necessity for processing undue numbers on a single day at the end of the feeding period.

Preliminary preparation of the tooth

At the conclusion of its 42-day feeding period each animal is sacrificed by chloroform. Its lower jaw is removed and divided by a vertical incision between the incisors. The exposed portions of the incisor and that portion of the mandible extending beyond the molars are clipped off (thus allowing the fixing agent more easily to reach the pulp of the incisor), and the remainder placed in 10% formalin.

Following a minimum fixation period of 48 hours in 10% formalin, the teeth are washed in 70% alcohol for 24 hours. Decalcification [3] is then carried out in 10% nitric acid, changing the acid every second day. In 36 to 48 hours the unwanted molar and a jaw tissue is readily trimmed away. The tooth is tested with a sharp needle and is removed from the acid when the entire specimen can be pierced easily. Complete decalcification is usually accomplished in 3 to 4 days.

After rinsing in 1 or 2 changes of water, the teeth are placed in 2% potassium alum for 12 hours. This is followed by another rinse in water and a transfer to 5% sodium bicarbonate for 24 hours.

The incisors are then thoroughly washed in running water for 12 to 24 hours, using a washing bobber, and are prepared for embedding in the steps indicated in table 3.

[3] Based on method published by F. W. Gairns. Stain Technology, vol. 19, no. 4, 1944.

The teeth are now orientated for longitudinal sectioning, embedded in new 60°C. paraffin, and cooled.

Cutting. The paraffin block containing the embedded tooth is secured in the microtome jaw and sections removed until the center of the tooth is exposed, i.e., to the point where the pulp cavity ceases to increase in length or width. From the ribbon of sections representing the tooth center, 4 to 6 sections of 8 to 10 microns in thickness are selected for microscopic examination.

TABLE 3

Steps through alcohol to paraffin.

MATERIAL	IN	OUT
10% alcohol [1]	9:00 a.m.	12:00 noon
20% alcohol [1]	12:00 noon	5:00 p.m.
40% alcohol [1]	5:00 p.m.	9:00 a.m.
60% alcohol [1]	9:00 a.m.	12:00 noon
80% alcohol [1]	12:00 noon	5:00 p.m.
Absolute alcohol	5:00 p.m.	9:00 a.m.
Absolute alcohol	9:00 a.m.	10:30 a.m.
Cedarwood oil	10:30 a.m.	12:00 noon
52°C. paraffin	12:00 noon	2:30 p.m.
60°C. paraffin	2:30 p.m.	4:30 p.m.
	changing once	

[1] Phenol may be added to these alcohols up to 6% to impart an elasticity to the tissues, thus facilitating the cutting of thin sections.

Chilling both knife and blocks in ice water for 10 minutes before sectioning increases the ease with which sections are cut.

Staining. Erhlich's acid haematoxylin and the counterstain eosin are employed in ordinary progressive staining.

The odontoblast row of each of the sections on the slide is examined and 5 readings are taken from that section bearing the highest odontoblasts. The average of these is considered to represent the maximum height of the odontoblast cells of that animal.

Measurements are made under 440 magnification by means of an ocular micrometer and the readings are subsequently

converted to microns. Groups of cells of the same height in the central area of maximum odontoblast development are chosen. Measurements are not made in the regions of the dental papilla, nor at the biting end of the tooth where the odontoblasts are seen in their embryonic and senile states, respectively. On levels of ascorbic acid below 1.0 mg the odontoblast development is more irregular and the avoidance of single cell readings is not always possible.

Statistical treatment of the data

The feeding tests are designed to permit standard analysis of the variance of the data and the application of the factorial scheme for isolating individual treatment effects as described by Bliss and Marks ('39). As already mentioned, at least 3 levels of known and unknown are fed. The factorial analysis, however, is applied to the levels of unknown which lie within the limits of the linear response of the known. By this procedure, the difficulty with levels of unknowns which prove to lie outside the limits of linear response is avoided.

ACKNOWLEDGMENTS

The series of studies upon which this report is based was made possible through the generous financial assistance of the Department of Pensions and National Health, now the Department of National Health and Welfare of the Canadian Government. The tedious work involved in the conduct of the numerous assays and especially in the histology, was carried in turn by Barbara Collier, Dr. L. D. Woolsey, Muriel Hughes, Florence Farmer, Milton Bell and Barbara Burton, former graduate students in this Department. Their assistance is gratefully acknowledged.

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THE RENAL THRESHOLD FOR ASCORBIC ACID

A MODIFIED METHOD FOR ITS ESTIMATION WITH RESULTS FOR TWELVE ADULT SUBJECTS [1]

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A method for estimating the renal threshold for ascorbic acid has been reported from this laboratory by Lewis, Storvick and Hauck ('43). A majority of their subjects were studied for 2-week periods, with daily determinations of fasting plasma ascorbic acid and corresponding 1-hour urinary excretion values. In later studies in this laboratory, such determinations were made daily for 6 weeks. With a large number of fasting values, a statistically significant difference was sometimes found between 2 means, both of which were below the renal threshold as estimated by inspection of the arrayed data and scatter diagrams. This paper presents a modified method for treating such data, with values for renal thresholds for ascorbic acid for 12 adult subjects, 1 of whom was previously studied by Lewis, Storvick and Hauck ('43).

EXPERIMENTAL PROCEDURE

Personal data concerning the subjects, the diets and experimental procedure are reported in connection with the studies

[1] Some of the data in this paper are taken from theses presented to the faculty of the Graduate School of Cornell University in fulfillment of the requirement for the M.S. degree, by Ann C. Moore, June 1944, and J. Estelle Haines, February 1946. Further details will be found in the theses.

on tissue reserves of ascorbic acid at various intakes (Haines et al., '47).

Blood and urine samples to be used in estimating the renal threshold were obtained as described by Lewis, Storvick and Hauck ('43) for their second series of experiments, i.e., the blood sample was taken midway in the hour for which the urine specimen was collected. Some of these specimens were obtained under fasting conditions, and some after various sized doses of ascorbic acid.

METHOD OF ESTIMATING THE RENAL THRESHOLD

In our modified method, inspection of the means and standard deviations for hourly urinary excretion of ascorbic acid at various plasma levels is substituted for Student's ('25) "t" test for determining the mathematical significance of the differences between means. The first step was to array the data for plasma ascorbic acid values for each subject, with the corresponding hourly urinary excretion values. Scatter diagrams were also made from these data. From inspection the apparent renal threshold for ascorbic acid was noted for each subject. The mean value, with its standard deviation, for urinary excretion of ascorbic acid at the apparent renal threshold was then calculated. This was compared with the mean value and its standard deviation for urinary excretions at all plasma levels below the apparent renal threshold. Since by definition, the renal threshold for ascorbic acid is the plasma concentration at which a marked rise in urinary excretion of ascorbic acid occurs, if the threshold value obtained by inspection is correct, the excretion of ascorbic acid at this plasma value will be relatively large and variable. Below the renal threshold, the excretion is low and relatively constant (Friedman, Sherry and Ralli, '40). To test the validity of the apparent renal threshold, similar calculations were made for plasma values 0.1 mg % below and 0.1 mg % above the apparent renal threshold.

RESULTS AND DISCUSSION

Renal threshold values for 12 subjects

Mean urinary excretion values for ascorbic acid corresponding to plasma values at, above and below the renal thresholds for 12 subjects are presented in table 1. For these subjects, the renal threshold values for plasma ascorbic acid as estimated by this method, ranged from 1.0 to 1.3 mg %. Mean excretion values for ascorbic acid at the threshold were all above 2 mg per hour, with most standard deviations considerably more than half the mean.

Of the renal thresholds for ascorbic acid reported in this paper and that of Lewis, Storvick and Hauck ('43), for 23 individuals, 21 were between 1.0 and 1.3 mg %. Inspection of the data for the entire series suggests that if a subject excretes 2 mg or more of ascorbic acid in 1 hour, his plasma ascorbic acid value has almost certainly exceeded his renal threshold during that hour, whereas if the excretion is 1.0 mg or less, it probably has not.

Comparison of the modified method for estimating renal threshold for ascorbic acid with that of Lewis, Storvick and Hauck ('43)

When the method described in this paper was applied to the data of Lewis, Storvick and Hauck ('43), the estimated renal thresholds were the same in all cases. When the method of Lewis, Storvick and Hauck was applied to data for the subjects of the present study, odds for significance for points below the apparent renal threshold were more comparable with those of Lewis, Storvick and Hauck when the fasting plasma values used in the calculations were limited to 2 weeks of the study. Thus the method presented here appears to have a wider usefulness than that previously devised in this laboratory.

Stability of the renal threshold for ascorbic acid

In the present study, 4 subjects, E.K., D.L., H.G. and A.M., were subjects for 2 successive years. Subject J.M. served

TABLE 1

Mean urinary excretion values for ascorbic acid corresponding to plasma values at, above, and below the renal threshold for ascorbic acid. (12 subjects.)[1]

SUBJECT	RENAL THRESHOLD	HOURLY EXCRETION AT THRESHOLD	HOURLY EXCRETION BELOW THRESHOLD	PLASMA VALUE BELOW THRESHOLD	HOURLY EXCRETION AROUND POINT TESTED	HOURLY EXCRETION BELOW POINT TESTED	PLASMA VALUE ABOVE THRESHOLD	HOURLY EXCRETION AROUND POINT TESTED	HOURLY EXCRETION BELOW POINT TESTED
	mg %	*mg*	*mg*	*mg %*	*mg*	*mg*	*mg %*	*mg*	*mg*
M. H.[2]	1.0	2.6 ± 1.3 (5)[3]	0.7 ± 0.4 (7)	0.9	1.4 ± 0.6 (2)	0.6 ± 0.2 (15)	1.1	2.4 ± 0.8 (5)	1.1 ± 1.1 (22)
J.L.	1.0	3.3 ± 1.9 (4)	1.1 ± 0.6 (38)	0.9	1.7 ± 0.7 (6)	1.0 ± 0.5 (32)	1.1	3.1 ± 1.9 (3)	1.2 ± 0.9 (41)
P.H.	1.05	5.7 ± 7.6 (4)	0.9 ± 0.4 (38)	0.95	1.3 ± 0.7 (5)	0.8 ± 0.3 (33)	1.15	7.8 ± 7.1 (4)	1.3 ± 2.5 (42)
E.K.	1.1	2.8 ± 2.0 (5)	1.4 ± 0.6 (46)	1.0	1.9 ± 0.8 (6)	1.3 ± 0.5 (40)	1.2	2.8 ± 1.5 (7)	1.3 ± 0.6 (46)
H.G.	1.1	2.1 ± 1.6 (7)	1.0 ± 0.3 (24)	0.95	1.3 ± 0.4 (3)	1.0 ± 0.2 (21)	1.25	3.4 ± 3.5 (6)	1.2 ± 0.9 (31)
J.M.[4]	1.1	3.2 ± 4.3 (9)	0.9 ± 0.5 (32)	1.0	0.9 ± 0.3 (6)	0.9 ± 0.5 (29)	1.2	8.3 ± 6.3 (5)	0.9 ± 0.4 (37)
D.L.[2]	1.15	4.2 ± 3.7 (5)	0.8 ± 0.3 (38)	1.05	1.3 ± 0.6 (3)	0.8 ± 0.2 (35)	1.25	2.7 (1)	1.2 ± 1.5 (43)
A.M.	1.2	7.7 ± 7.1 (3)	0.8 ± 0.4 (43)	1.1	1.2 ± 0.8 (6)	0.7 ± 0.2 (37)	1.3	4.8 ± 5.7 (6)	1.2 ± 2.3 (46)
L.G.	1.3	6.5 ± 4.3 (7)	1.0 ± 0.4 (27)	1.2	1.2 (1)	1.0 ± 0.4 (26)	1.4	6.3 ± 0.1 (2)	2.2 ± 3.0 (34)
J.E.S.[2]	1.3	2.8 ± 1.6 (3)	1.1 ± 0.5 (21)	1.2	1.9 ± 0.2 (2)	1.0 ± 0.4 (19)	1.4	2.5 ± 1.7 (6)	1.3 ± 0.9 (24)
E.H.	1.3	3.3 ± 2.3 (8)	1.0 ± 0.5 (28)	1.2	1.1 ± 0.4 (4)	1.0 ± 0.6 (24)	1.4	6.8 ± 1.2 (4)	1.5 ± 1.5 (36)
S.P.	1.3	2.2 (1)	0.9 ± 0.3 (30)	1.2	1.1 ± 0.2 (4)	0.8 ± 0.3 (26)	1.4	4.8 ± 0.2 (2)	0.9 ± 0.4 (41)

[1] When a subject was studied during 2 successive years, values for the larger series of the 2 are given.

[2] Some of the determinations used in calculating the renal threshold for these subjects were made by Margaret Delaney.

[3] Figures in parentheses represent number of values included in the mean.

[4] This subject also served for the experiments of Lewis, Storvick and Hauck ('43).

for this experiment and also that of Lewis, Storvick and Hauck ('43). These authors also reported the renal threshold for 1 subject (J.S.) who served for 2 successive years. Whether values for these subjects were calculated for the separate years or combined, the estimated renal thresholds were the same. Evidence from these 6 subjects suggests, therefore, that the renal threshold for ascorbic acid is relatively stable in the normal adult from year to year, and, within the limits of this experiment, at various levels of intake.

SUMMARY

A modified method for estimating the renal threshold for ascorbic acid based on the comparison of means and standard deviations for urinary excretions at various plasma ascorbic acid levels is presented. This method is simpler and appears to have a wider usefulness than that previously reported from this laboratory.

The renal thresholds for 12 normal adults, as estimated by this method, were between 1.0 and 1.3 mg %.

Further evidence is presented that the renal threshold for ascorbic acid is relatively stable from year to year.

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* This subject also served for the experiments of Lewis, Storvick and Hauck ('43).

PLASMA LEVELS AND URINARY EXCRETION OF ASCORBIC ACID IN WOMEN DURING THE MENSTRUAL CYCLE [1]

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Variations in the urinary excretion of ascorbic acid (Fernandes, '39; Pillay, '40; Mickelson et al., '43; Hartzler, '45) and in fasting plasma ascorbic acid values (Mickelson et al., '43) in relation to the menstrual cycle, have been reported, but these reports are not in agreement. Fernandez ('39) observed "inconceivably high values" for dye reduction by urine just before menstruation, whereas Hartzler noted low values for urinary excretion of ascorbic acid just before and/or during the early part of the menstrual period. Pillay ('40) found the lowest excretion of ascorbic acid after a test dose at the midpoint of the menstrual cycle, whereas Mickelson et al. ('43) found "a sharp increase in the fasting level of plasma vitamin C" in some women during the middle of the menstrual cycle, with some increase in vitamin C excretion on the days corresponding to this peak.

Some day-to-day variations occur in fasting plasma levels and urinary excretion of vitamin C even on a constant diet. Of the investigations mentioned, however, only 2 were during controlled vitamin C intake, namely that of Hartzler ('45) in which the variations with menstruation were noted in 1 series

[1] Most of the vitamin C determinations were made by Clara A. Storvick, Ann C. Moore, Alice Kline, Margaret Delaney and Estelle Haines in connection with Purnell projects directed by the author.

of experiments but not in another, and that of Mickelson et al. ('43) in which 1 of 8 subjects was kept on a constant vitamin C intake throughout 1 menstrual cycle.

Since, in the course of our studies on vitamin C metabolism, a number of women subjects have been maintained on a constant level of vitamin C intake for periods of 4 and 6 weeks, the data obtained on fasting plasma values and urinary excretion of vitamin C were examined to see if any relation to the menstrual cycle could be noted.

EXPERIMENTAL

Ten women subjects served during experimental periods of 4 or 6 weeks, i.e., enough to include 1 menstrual cycle. In all, data were available for 30 menstrual periods. In 10 instances 2 menstrual periods occurred in the course of an experiment, so that the urinary excretion and the plasma ascorbic acid values could be noted for the midpoint of the cycle.

Details as to the experimental procedures and diet are reported elsewhere (Belser, Hauck and Storvick, '39; Storvick and Hauck, '42; Haines et al., '47). The results are presented in table 1. When a menstrual period occurred at the beginning or end of an experiment, values for those days during the presaturation or resaturation periods were not included, i.e., all values shown in table 1 are for days or periods on the constant level of intake stated.

Since all of the experiments began with a period of high intake of ascorbic acid to assure saturation, there was some downward trend in urinary excretion and plasma ascorbic acid values for all subsequent intakes. The week of the experiment during which the menstrual period occurred is indicated since, in general, weekly means for the early part of the experiment were above the general mean, whereas those toward the end of the experiment were below it. Most instances in which the values related to the menstrual period differed from the mean by more than the standard deviation occurred toward the beginning or end of the experiment, and this circumstance, rather than the associated menstrual period,

TABLE 1

Plasma values and urinary excretion of ascorbic acid in relation to the menstrual period.

SUBJECT	LEVEL OF INTAKE, MG	WEEK [1]	ASCORBIC ACID IN URINE, MG						PLASMA ASCORBIC ACID VALUES, MG %	
			Mean for expt.[2]	Day before M.P.	First day of M.P.	Mean for M.P.	Mid-value [3]	Week of mid-value [4]	Mean for expt.[2]	Mid-value [3]
D.L.	53	3	23 ± 6	22	22	22				
	50	3	18 ± 4	16	18	18				
J.E.S.	50	2	21 ± 4	25	22	19	17	4	0.9 ± 0.2	0.8
	50	6	21 ± 4	*15*	*16*	*16*				
M.H.	50	3	18 ± 5	20	16	17	17	4	0.7 ± 0.1	0.6
	50	6	18 ± 5	15	16	[5]				
E.H.	33	4	25 ± 4	23	*30*	26	26	2	0.5 ± 0.2	0.7
L.G.	33	3	19 ± 3	21	20	21	18	5	0.5 ± 0.2	0.4
A.M.	70	3	24 ± 6	20	21	22	18	4	0.9 ± 0.1	0.8
	70	6	24 ± 6	18	26	23 [6]				
	53	4	19 ± 3	18	18	18	20	2	0.8 ± 0.2	0.9
C.S.	210	3	154 ± 18	148	140	146				
	135	1	82 ± 10	90	*97*	85				
	110	1	59 ± 10	68	64	62				
	100	3	49 ± 20	30	31	35				
	65	2	29 ± 8	26	29	26				
K.J.	210	2	164 ± 14	153	171	162				
	85	1	45 ± 10	*75*	*64*	*59*	37	2	1.2 ± 0.1	1.1
	85	4	45 ± 10	35	40	[5]				
	75	3	38 ± 11	44	35	38				
H.H.	210	3	163 ± 13	165	*144*	160				
	135	2	87 ± 11	93	[7]	96 [7]				
	110	3	65 ± 12	70	61	64				
	100	1	58 ± 13	*162*	*110*	*90*	50	2	0.8 ± 0.1	*0.6*
	100	4	58 ± 13	58	52	[5]				
	60	2	26 ± 11	26	30	25				
J.S.	210	1	158 ± 14	*226*	*175*	*184*	154	2	1.3 ± 0.1	1.3
	210	4	158 ± 14	164	151	149				
	60	1	26 ± 9	[8]	*170*	*89*	30	2	1.0 ± 0.1	1.0
	60	4	26 ± 9	21	21	20 [6]				

[1] Week of experiment in which menstrual period started.
[2] Values for first 2 days of experimental period omitted in calculating the general mean.
[3] Value for mid-point of menstrual cycle.
[4] Week of experiment in which mid-point of menstrual cycle occurred.
[5] One day only on constant intake.
[6] Two days only on constant intake.
[7] Value not obtained on first day of menstrual period.
[8] During presaturation period.

Values which differ from the mean value for the period by more than the standard deviation have been italicized.

might account for the deviation from the mean value for the period. Note, for example, that of 30 urinary excretion values, obtained on the day before a menstrual period only 4 differed from the mean value by more than the standard deviation. The 3 instances in which the differences were positive, all occurred during the first week of the experiment, whereas the 1 instance in which the difference was negative occurred in the last week of the experiment. Similarly, few marked variations from the means were noted among urinary excretion values for the first day or for the entire menstrual period. In most of these instances, the downward trend in urinary excretion values for vitamin C, rather than menstruation, might account for the unusual values.

Of 10 plasma ascorbic acid values obtained at the mid-menstrual period for 8 women, only 1 differed from the mean for the experiment by more than the standard deviation. In no instance was there an unusual ascorbic acid excretion at the mid-menstrual period.

Not only were unusual variations in urinary excretion of ascorbic acid at the menstrual period the exception rather than the rule, but such unusual variations as were noted were not consistently in 1 direction. Equally great variations, both positive and negative, have been noted in excretion values for our male subjects.

SUMMARY

In 10 women subjects whose ascorbic acid metabolism was studied for a total of 23 periods of from 4 to 6 weeks duration, during which 30 menstrual periods occurred, no evidence was found of unusual variability in either urinary excretion of ascorbic acid or fasting plasma ascorbic acid values associated with the menstrual cycle.

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BIOTIN BALANCE IN THE ALBINO RAT[1]

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The theory that "avidin" (Eakin et al., '40, '41) or "antibiotin" (Woolley and Longsworth, '42) of raw egg-white combines with biotin in the digestive tract making biotin unavailable for absorption and resulting in an induced deficiency (Parsons et al., '40; György et al., '41) is now well accepted. Du Vigneaud et al. ('42) have suggested that the urea grouping of biotin is essential for the combination of biotin with avidin. The present study was initiated in an attempt to obtain additional evidence on the biotin-avidin interrelationship in the digestive tract by means of biotin balances. The experimental data were obtained during the years 1940–1942 but because of unforseen circumstances publication has been delayed until the present time.

EXPERIMENTAL

The fate of the biotin in the presence and absence of egg-white was studied in young rats during induced deficiency and recovery periods, and in adult rats on rations with and without egg-white and a rich source of biotin.

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Rations

Rations containing either raw or thoroughly cooked dried egg-white were used in this study. Table 1 shows the levels at which the egg-white was included in the various rations. In 1 series (lots I, II, III, VII, VIII and IX), the other ingredients were (Osborne and Mendel, '19) salt mixture 4%, dried yeast 5%, wheat germ (or rice polishings) 10%, lard 5%, sugar (or starch) to make 100%. In another series (lots IV, V, VI, XI and XII) cooked pork kidney replaced the yeast, lard and wheat germ (or rice polishings) and the sugar or starch was adjusted accordingly. A third kind of ration (stock) consisting of casein 5%, dried yeast 3%, wheat germ 2.5%, yellow corn 50%, oil meal 11%, alfalfa meal 1.5%, butterfat 5%, cod liver oil 2.5%, $CaHPO_4 \cdot 2H_2O$, 1%, NaCl 0.5%, and dried milk 18% was fed to lots X and XIII.

Animals

Albino rats were prepared for the experiment by placing them at weaning time on a ration containing 20% dried raw egg-white. When they had regained their weaning weight, the amount of raw egg-white in the diet was increased to 40% and finally to 66%. Moderate to severe biotin deficiency manifestations appeared in about 50 days. The manifestations of the induced biotin deficiency on these rations have been described (Parsons, '31; Parsons et al., '37). Inasmuch as the deficiency was induced in rats on rations containing 20% of egg-white as well as on 66%, the higher protein concentration presents no important complication in an interpretation of the results. At this time, the animals were placed in metabolism cages and urine and feces collections were made over a 4-day period (lot I, table 1). To study further the biotin balance during the deficiency period, the rats were then divided at random into 3 groups. One group was continued on 66% raw egg-white, a second was placed on 30% raw dried egg-white (lot II) and the third was fed 30% cooked dried egg-white (lot III). After 15 to 25 days had elapsed, urine and

feces were collected from lots II and III. Following this, some of the animals of the 3 groups just mentioned were chosen at random, and cooked dried pork kidney, an excellent, natural source of biotin,[2] was incorporated in their ration at a level of 25%. These groups are designated as lots IV, V and VI, and were taken from lots I, II and III, respectively. Collections were made of the elimination products after a 7- to 10-day adjustment period.

It was thought that a group of adult animals with similar nutritional histories on egg-white rations would be valuable in showing the effect of high levels of egg-white in the diet on the fecal output even though urinary studies could not be included. Such a group of rats which had been kept on a 20% raw egg-white ration and which had reached a fairly constant level of biotin elimination was divided into 3 groups. One group was continued on the 20% raw dried egg-white ration (lot VII), the second group was placed on a ration containing 66% raw dried egg-white (lot VIII) and the third on 66% cooked dried egg-white (lot IX).

Adult rats on the stock ration (lot X) were used to indicate the biotin balance of animals on an egg-white-free ration.

In an attempt to find the response of normal rats to the presence of egg-white and biotin in the ration, 2 of the rats from lot X were placed on a ration containing 30% raw dried egg-white and 25% cooked pork kidney (lot XI), and 2 were fed a ration containing 30% cooked dried egg-white plus 25% cooked dried pork kidney (lot XII).

The biotin balances of the stock rats and of the rats on the various egg-white rations are summarized in table 1.

On completion of the collections of urine and feces, the animals were killed. The livers and kidneys were removed and analyzed to determine if the biotin content of these organs bore any relationship to biotin intake or output. The contents of the digestive tract — the stomach, small intestine, cecum,

[2] Pure biotin would have been preferable for this purpose, but, unfortunately, at the time the work was done (1940–1941) adequate supplies of crystalline material were not available.

and large intestine — were also assayed for biotin. Such results might give some indication of the site of avidin-biotin fixation, and provide evidence for or against biotin synthesis in the digestive tract with the possible location of that synthesis. Only 3 of the animals in each of lots VII and VIII were used for this part of the experiment. These animals form lots VIIa and VIIIa given in table 2. Lot VIa is made up of the animals in lot VI, table 1, and 1 additional rat of the same nutritional history. Lot XIII was composed of a group of adult rats on the stock ration. This was a separate group from lot X.

Assays

Urine and feces were collected separately, daily. The feces were dried at room temperature; the urine was preserved with toluene and chloroform and kept in the refrigerator. At the end of the 4-day period, composite samples were made.

Biotin was determined by the microbiological assays of Lampen et al. ('41) and of Snell et al. ('40). All biotin values are based on at least 3 levels of assay in which results agreed within 10%.

As biotin in urine is present in water-soluble form no further treatment of the urine samples was required. Feces, liver and kidney, and contents of the digestive tract, however, contain bound biotin and this was liberated by acid hydrolysis. One gm of the dry substance was suspended in 40 ml of 2 N H_2SO_4, autoclaved for 2 hours at 15 lbs. pressure, cooled, neutralized with NaOH, filtered, and made up to 50 ml. If not assayed immediately, the hydrolyzed samples were preserved with toluene and chloroform and stored in the cold room until assayed.

RESULTS AND DISCUSSION

Biotin balances of rats on stock ration

Normal adult rats on the stock ration (table 1, lot X) eliminated from 0.7 to 2.0 μg (average 1.1 μg) of biotin daily in the feces while the output in the urine ranged from 0.6 to 1.0 μg (average 0.8 μg). Thus there was an approximately equal

TABLE 1

Biotin balances of albino rats on stock and egg-white rations.

LOT	INGREDIENTS OF RATION		NUMBER OF RATS	AVERAGE DAILY FOOD INTAKE	AVERAGE DAILY BIOTIN INTAKE [2]	AVERAGE DAILY ELIMINATION OF BIOTIN [2,3]			RATIO OF AV. BIOTIN ELIMINATION TO INTAKE
	Kind [1]	Amount				Urine	Feces	Total	
		%		gm	μg	μg	μg	μg	
I	EWR	66	10	5.5	3.2	0.6	4.4	5.0	
					(2.6–3.7)	(0.1–1.2)	(2.8–6.1)	(2.8–7.3)	1.6
II	EWR	30	3	7.7	2.3	0.6	4.6	5.2	
					(2.1–2.4)	(0.5–0.8)	(2.7–8.0)	(3.3–8.5)	2.2
III	EWC	30	4	11.5	3.7	0.8	4.8	5.6	
					(3.2–4.1)	(0.7–1.2)	(2.9–5.5)	(3.6–6.7)	1.5
IV	EWR	66			15.3	1.1	23.6	24.7	
	PKC	25	3	13	(14.2–17.5)	(1.0–1.1)	(21.8–25.2)	(22.3–26.4)	1.6
V	EWR	30	2	16	16.9	3.2	14.3	17.5	
	PKC	25			(15.8–17.9)	(3.0–3.4)	(13.2–15.4)	(16.6–18.4)	1.0
VI	EWC	30	2	10	10.5	3.2	4.6	7.8	
	PKC	25			(9.5–11.6)	(3.0–3.3)	(3.1–6.1)	(6.1–9.4)	0.7
VII	EWR	20	11	6.0	1.6		3.2		
					(1.1–2.6)		(1.6–5.3)		
VIII	EWR	66	5	4.8	3.0		4.2		
					(1.2–3.8)		(1.3–3.8)		
IX	EWC	66	2	8.5	5.3		5.8		
					(5.0–5.6)		(5.0–6.7)		
X	Stock	100	4	9.8	1.7	0.8	1.1	1.9	
					(1.3–3.0)	(0.6–1.0)	(0.7–2.0)	(1.3–3.0)	1.1
XI	EWR	30	2	8	8.5	2.2	10.1	12.3	
	PKC	25			(7.4–9.5)	(2.0–2.4)	(9.9–10.3)	(12.0–12.7)	1.4
XII	EWC	30	2	8	8.5	2.6	4.4	7.0	
	PKC	25			(7.4–9.5)	(2.2–2.9)	(3.4–5.3)	(6.3–7.4)	0.8

[1] EWR, raw egg-white; EWC, cooked egg-white; PKC, cooked pork kidney.

[2] Figures in parentheses denote the range of biotin.

[3] Average of 4 days' collections.

distribution of the total biotin output between feces and urine.

Biotin balances of rats on egg-white and biotin-rich rations

According to the hypothesis that avidin fixes biotin in an unabsorbable combination, it would be expected that increasing the amount of raw egg-white in the ration would increase the biotin in the feces. The averages are in general agreement with this hypothesis. The biotin content of the feces from lots fed raw egg-white averaged 139% of the ingested biotin while the urine contained only 19%. Corresponding figures for lots fed cooked egg-white were 75% and 28%, respectively. The distribution in the latter group resembles that found in the stock ration group.

At the high levels of biotin in the ration, its fixation by avidin is especially apparent. The average fecal biotin on the raw egg-white rations containing cooked kidney exceeded by 3- to 5-fold the corresponding average biotin elimination on rations containing egg-white without kidney (lots I and IV; II and V). Likewise, the fecal biotin output was 2 to 3 times as much on the raw egg-white ration with pork kidney as on the cooked egg-white and pork kidney rations (lots V and VI; XI and XII). These data support the view that the avidin of thoroughly cooked egg-white does not retain its biotin-binding property.

The response of adult stock rats (lot X) to egg-white and high levels of biotin (lots XI and XII) was very marked. Both the urinary and fecal biotin eliminations were increased. With raw egg-white in the ration, the fecal biotin was 10-fold that on the stock ration, while a 4-fold increase occurred on the ration containing cooked egg-white.

There is clear evidence that biotin from some non-dietary source was eliminated in the feces of rats on egg-white rations. It will be noted (table 1) that the ratio of total daily biotin output to intake on most of the egg-white rations exceeded 1, and that 60 to 95% of the output was present in the feces.

The biotin in the urines, in contrast, represented only 5 to 40% of the daily biotin intake. The probable explanation is a synthesis of biotin somewhere in the digestive tract. The bacteria native to the intestinal tract are known to synthesize biotin (Landy et al., '42; Thompson, '42; Burkholder and McVeigh, '42). Direct evidence has also been reported to show that biotin synthesis occurs in animals (McElroy and Jukes, '40; Wegner et al., '41; Nielsen et al., '42; Mitchell and Isbell, '42) and in human subjects (Gardner et al., '43, '45, '46; Oppel, '42; Parsons et al., '42).

An indication of the site of the biotin synthesis is given by the amount of biotin found in the contents of the stomach, small intestine, cecum and large intestine. From table 2, it is evident that the biotin concentration in the cecum and large intestine was strikingly greater than that in the stomach and small intestine. This increase was as much as 3- to 20-fold with respect to the rats on the egg-white rations. The cecum and large intestine of stock rats contained 2.0 μg and 2.3 μg per gm of dry matter, respectively, as compared with 1.0 and 0.9 μg in the stomach and small intestine. Based on the total contents (see footnote 1, table 2) one-third of the total biotin in the tract was found in the cecum and large intestine. Likewise with egg-white in the ration, from 88–98% of the total biotin was present in the contents of these 2 sections.

The presence of large amounts of biotin in the ceca suggests biotin synthesis at this point in the digestive tracts. Mitchell and Isbell ('42) have presented data which show that intestinal flora in the cecum may contribute largely to the rat's supply of several of the B-vitamins and indicate that these vitamins are absorbed by the body tissues.

Another possible source of non-dietary fecal biotin is the withdrawal and elimination of body stores of biotin. From the standpoint of the biotin concentration in the livers and kidneys (table 2) there is obviously no evidence of withdrawal of body stores due to high levels of raw egg-white. The excess of biotin output over intake for long periods of

TABLE 2

Biotin concentration in the contents of the digestive tract and in the organs and feces of albino rats on stock and egg-white rations.

LOT	BIOTIN IN RATION	BIOTIN IN THE CONTENTS OF THE DIGESTIVE TRACT AND IN THE ORGANS [1]						BIOTIN IN FECES PER DAY	
		Number of rats	Stomach	Small intestine	Cecum	Large intestine	Livers and kidneys	No. of rats	Biotin
	μg/gm		*μg/gm*	*μg/gm*	*μg/gm*	*μg/gm*	*μg/gm*		*μg/gm*
IV	1.2	3	2.2 (2.1–2.3)	6.2 (1.0–9.4)	20.0 (10.1–32.1)	41.1 (32.2–50.0)	1.1 (0.8–1.4)	3	33.8 (30.6–38.7)
V	1.1	2	2.6 (0.6–4.6)	4.9 (4.7–5.1)	14.0 (13.6–14.4)	15.7 (10.5–20.9)	2.7 (1.9–3.4)	2	16.6 (15.3–17.9)
VIa	1.1	3	1.3 (0.8–1.7)	1.5 (0.5–3.5)	5.6 (4.2–6.3)	7.1 (4.1–9.1)	1.4 (1.2–1.6)	2	9.3 (7.6–11.0)
VIIa	0.3	3	0.8 (0.2–1.3)	1.3 (1.0–1.4)	9.7 (8.4–11.8)	10.2 (6.8–13.5)	1.0 [2]	11	7.4 (3.6–12.6)
VIIIa	0.6	3	0.5 (0.3–0.7)	1.8 (0.3–3.0)	12.9 (10.7–14.0)	12.9 (11.4–14.4)	6.0 (1.3–16.3)	5	10.2 (8.0–15.4)
X and XIII [3]	0.2	9	1.00 (0.3–1.3)	0.9 (0.2–2.0)	2.0 (1.2–3.2)	2.3 (1.6–3.8)	0.9 (0.6–1.0)	4	1.1 (0.8–1.3)

[1] Average total weight of dry material as follows: contents of stomach 0.6 gm; small intestine 0.8 gm; cecum 0.5 gm; large intestine 0.4 gm; livers and kidneys lot IV 2 gm; V 2.7 gm; VI 2.8 gm; VIIa 2 gm; VIIIa 1.6 gm; XIII 4.0 gm.

[2] Liver and kidneys for 1 rat only.

[3] Lot X was composed of 4 animals, lot XIII of 5.

time is too great to assume that it could have originated from body stores.

The data in table 2 also give some indication of the comparative absorbability of biotin and the other constituents of the ration. As would be expected if the biotin-avidin complex is unavailable, an increase was found in the biotin concentration of the contents of the small intestine over the contents of the stomach and over that of the ration. This is particularly evident on raw egg-white rations containing cooked pork kidney (lots IV and V). The data indicate that part of the ingredients of the ration was absorbed from the small intestine faster than was the biotin; especially was this true where large amounts of biotin were present in a presumably unabsorbable form. Since synthesis of the B-vitamins is believed to occur in the digestive tract at some place beyond the small intestine, this increase in the small intestine is probably not accounted for by biotin synthesis.

SUMMARY

Evidence is presented that biotin from some non-dietary source was eliminated in the feces of rats on rations containing raw egg-white. The excess biotin in the urine and feces over that contained in the ration ingested ranged from 0.6 μg to 9.4 μg per day. The excess biotin presumably originated from synthesis of biotin by intestinal microorganisms rather than from the withdrawal of biotin stores in the liver, kidney or other body tissues. The main site of synthesis in the digestive tract appeared to be the cecum and large intestine.

Of the total biotin eliminated from 5 to 40% was found in the urine and from 60 to 95% was present in the feces.

The fixation of biotin by avidin was more apparent at the higher levels of biotin in the ration.

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A STUDY OF ASCORBIC ACID METABOLISM OF ADOLESCENT CHILDREN [1]

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Studies of the human requirement for ascorbic acid have been made with adults and with children up to 12 years of age. As far as we have been able to ascertain, the requirements of adolescents have not been determined experimentally. This study was undertaken to provide some quantitative data for the estimation of the ascorbic acid need of children during early adolescence.

Children living at the Children's Farm Home, Corvallis, Oregon, served as subjects. These children, about 160 in number and ranging in age from 5 to 18 years, are housed in 8 cottages at the Children's Farm Home. Boys and girls live at separate cottages, except at the "health cottage," where a registered nurse resides, and which houses convalescents and children requiring special care. In order to simulate family life, children of different ages live in each cottage, and each cottage has its own cook and house-mother. All food is supplied through a central commissary, but its use depends upon the choices of the cooks.

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A survey of the plasma ascorbic acid values of 81 children between the ages of 11 and 18 was made. Eight of these children, aged 12 to 14 years, also served as subjects in an experimental study. Three levels of ascorbic acid intake were tested for 1 week each with all subjects. One of the main purposes of the experiment was to study the effect on the plasma ascorbic acid concentration of the daily allowance of ascorbic acid which has been recommended by the National Research Council ('45).

SURVEY OF PLASMA ASCORBIC ACID VALUES OF ADOLESCENTS

Throughout the entire study the determination of reduced ascorbic acid in plasma was made by the micro-method of Farmer and Abt ('36), using samples of blood obtained by fingerprick. During the survey, the blood samples were obtained either before breakfast or just before lunch if the

TABLE 1

A summary of the plasma ascorbic acid determination on adolescents at the Children's Farm Home.

AGE	NUMBER OF CHILDREN	MEAN	AVERAGE DEVIATION	RANGE
		mg %	*mg %*	*mg %*
11	10	0.60	0.15	0.31–0.97
12	19	0.53	0.22	0.17–0.95
13	21	0.62	0.20	0.08–1.14
14	18	0.56	0.16	0.06–1.07
15	6	0.43	0.13	0.26–0.61
16	4	0.62	0.14	0.51–0.90
17	3	0.72	0.15	0.50–0.84

breakfast had contained no fruit. Values for plasma ascorbic acid were obtained from 43 boys and 38 girls between the ages of 11 and 18. The results in terms of mean, average deviation, and range are summarized for each age in table 1.

The Committee on Vitamins of the American Academy of Pediatrics, headed by Allan M. Butler ('40), reports that a plasma ascorbic acid value of 0.60 mg % may be regarded

as adequate. Seventy-one per cent (27) of the plasma levels of the girls and 42% (18) of those of the boys of the Children's Farm House fell below this level. The mean plasma value for the entire group tested, however, was 0.56 mg %, which very nearly meets this standard.

EXPERIMENTAL STUDY

The experimental study was designed to compare the daily values for ascorbic acid in the plasma of adolescents when on their usual diet, when saturated with ascorbic acid, and when receiving the daily allowance of ascorbic acid which has been recommended by the National Research Council ('45). A description of the subjects is included in table 2. The girls

TABLE 2

Description of experimental subjects.

SUBJECT	AGE		HEIGHT	MEAN WEIGHT DURING STUDY
	years	*months*	*inches*	*lbs.*
Raymond	13	5	62.0	98
James	12	11	64.0	95
Jack	12	4	60.0	86
Jim	12	4	61.5	82
Jo	14	6	64.0	134
Stasia	13	9	65.5	101
Betty Lou	13	8	64.5	110
Sharon	12	3	62.0	107

lived at one cottage, and the boys lived at another. All of the children were apparently healthy, and examination of their medical records showed no evidence of abnormalities.

All of the foods which the subjects ate were weighed and recorded during a period of 3 weeks for each group. Samples of foods which might be presumed to contain ascorbic acid, were analyzed [3] after each meal by the method of Loeffler and Ponting ('42). Throughout the experimental study, blood samples were taken daily before breakfast.

[3] Details of the experimental methods and procedures, as well as complete tables of the results of food analyses, will be available as a technical bulletin published by the Oregon Agricultural Experiment Station.

Each experimental period of 3 weeks was divided into 3 parts. During the first week, the children ate the regular diet which was served in their cottage, the second week served as a saturation period, and during the third week, each child received the total amount of ascorbic acid recommended for his age and sex by the National Research Council ('45).

During the week on their regular diet, the first serving of each food was usually the same for each child, and if quantity permitted, it was a "standard" serving. During this week the children were allowed to have second servings of any food which they requested, as long as the supply lasted. Ascorbic acid intake varied, as did the intake of all other constituents during this week, according to the inclination of each child. In this way the effect of the diet, as usually served and eaten, on the ascorbic acid in the blood plasma, could be observed.

The second week served as a saturation period. In addition to the regular diet, from which significant sources of ascorbic acid were removed and replaced by foods of low ascorbic acid content, the children received a supplement of 200 mg of pure ascorbic acid [3] just before breakfast.

During the third week the ascorbic acid allowed from food was limited to 20 mg per day. Pure ascorbic acid was given before breakfast, as the saturation supplement had been, in order to bring the total daily intake to the amount recommended by the National Research Council ('45). The girls received a total of 80 mg of ascorbic acid daily: 20 mg from food and 60 mg as crystalline ascorbic acid dissolved in redistilled water. Using the age groupings of the National Research Council ('45), the boys fell into different groups. The fraternal twins, Jack and Jim, who had recently passed their twelfth birthday, received 55 mg of ascorbic acid plus 20 mg in food making their total daily intake 75 mg. The older boys, James (12 years and 11 months old) and Raymond (13 years, 5 months) received a supplement of 70 mg of the crystalline ascorbic acid or a total daily intake of 90 mg.

[3] Acknowledgment is made to Merck and Company, Inc., for a generous supply of crystalline ascorbic acid.

Although all foods were weighed, the children were allowed to eat, ad libitum, bread, butter, meat, and other foods which contained no ascorbic acid.

Between-meal eating presented some problems. The girls were accustomed to receiving some food after school so they were provided with extra food in the afternoon. The director of the boys' cottage, however, did not permit between-meal eating, so no after-school lunch was provided for them. The investigators felt that they had the complete cooperation of the subjects during the course of the experiment.

RESULTS OF EXPERIMENTAL STUDY

Table 3 presents the results of daily fasting plasma ascorbic acid determinations, and the corresponding daily intakes of each child during the study. Since no effort was made to change the diet of the children during the first period, the means for the entire period are reported, with the average deviations from the mean, as well as the range of values obtained during the period. The first 2 days were omitted from the calculation of means for the saturation periods and of the period when the recommended allowance of the National Research Council ('45) was given, to eliminate the effect of the previous level of intake. Average deviations from the means for the last 5 days of these 2 periods as well as the range of values are reported with the means for each subject.

The first period of observation with the boys showed them to be receiving a mean daily ascorbic acid intake of 61 mg, and maintaining blood plasma levels well within the range of adequacy, according to Butler ('40). Daily variations were rather marked throughout the study for most of the boys. James, however, varied little from day to day except during the saturation week. In spite of the daily variation, some general trends are apparent. There was a slight but definite increase in blood plasma ascorbic acid during the saturation period for each boy, without exception. The plasma levels of all of the boys dropped immediately with the lowered intake of ascorbic acid on the fifteenth day, and except for the daily

TABLE 3

Total daily intake and plasma ascorbic acid values for all subjects during 3 consecutive 1-week experimental periods.

SUBJECT	WEEK OF USUAL DIET MEAN ASCORBIC ACID		SATURATION WEEK ASCORBIC ACID			LAST 5 DAYS OF N.R.C. RECOMMENDED ALLOWANCE MEAN ASCORBIC ACID		
	Intake	Plasma	Mean intake	Mean plasma	Highest plasma value	Intake	Intake	Plasma
	mg/day	*mg %*	*mg/day*	*mg %*	*mg %*	*mg/day*	*mg/kg*	*mg %*
Raymond	59.1 ± 25.0 [1] (13–104) [2]	0.78 ± 0.10 (0.57–1.04)	233.8 ± 3.8 (227–243)	1.48 ± 0.19 (1.21–1.81)	1.37 [3]	90.0 ± 0.0	2.0	0.91 ± 0.05 (0.85–0.99)
James	62.1 ± 25.6 (13–114)	0.73 ± 0.03 (0.66–0.80)	236.2 ± 5.4 (227–243)	1.05 ± 0.08 (0.94–1.17)	1.17	90.0 ± 0.0	2.1	0.84 ± 0.03 (0.80–0.91)
Jack	60.3 ± 24.6 (13–104)	0.75 ± 0.09 (0.53–0.88)	236.2 ± 5.4 (227–243)	1.05 ± 0.03 (0.98–1.10)	1.10	75.0 ± 0.0	1.9	0.88 ± 0.07 (0.78–1.01)
Jim	62.0 ± 24.6 (13– 94)	0.75 ± 0.09 (0.49–0.97)	234.8 ± 5.0 (227-243)	1.19 ± 0.05 (1.07–1.25)	1.25	75.0 ± 0.0	2.0	0.98 ± 0.06 (0.86–1.03)
Jo	50.1 ± 15.9 (21– 88)	0.44 ± 0.06 (0.29–0.54)	215.4 ± 5.3 (207–223)	0.92 ± 0.15 (0.71–1.06)	1.06	80.0 ± 0.4 (79–81)	1.3	0.89 ±0.03 (0.84–0.96)
Stasia	41.6 ± 10.9 (24– 62)	0.47 ± 0.06 (0.28–0.59)	216.4 ± 4.5 (210–223)	0.96 ± 0.08 (0.84–1.13)	1.13	80.0 ± 0.4 (79–81)	1.7	0.86 ± 0.07 (0.74–0.94)
Betty Lou	42.9 ± 9.6 (29– 61)	0.23 ± 0.03 (0.18–0.29)	216.4 ± 4.5 (210–223)	1.14 ± 0.10 (0.89–1.35)	1.35	80.0 ± 0.4 (79–81)	1.6	1.21 ± 0.06 (1.13–1.35)
Sharon	47.0 ± 14.9 (25– 76)	0.20 ± 0.04 (0.13–0.31)	217.0 ± 5.2 (210–224)	0.97 ± 0.08 (0.76–1.03)	1.03	80.0 ± 0.4 (79–81)	1.6	0.91 ± 0.02 (0.86–0.95)

[1] Average deviation.
[2] Range.
[3] Raymond's value before illness.

variations, they remained at levels lower than their saturation levels, varying around 0.9 mg %.

During the third period when the subjects were in 2 age groupings, no difference was observed between the plasma levels for the 13-year-old boys receiving 90 mg per day and the 12-year-old boys receiving 75 mg per day.

The outstanding feature of this period is the peak reached by Raymond on the fourteenth day. He had been found to have a fever of 102½°F. after supper on the twelfth day. He was given 2 tablets of aspirin and 1 tablespoon of citro-carbonate by the house-mother, and he did not report for a blood sample the next morning. He remained in bed with a slight fever during that day, although he was kept as a subject. The next morning his plasma level had reached 1.81 mg %. Raymond had no fever during that day, and the following morning his plasma level was still much higher than that of the other children, namely, 1.54 mg %.

The possibility that the ingested aspirin or citro-carbonate might have caused the rise in Raymond's plasma value was considered. However, no increase in the concentration of ascorbic acid was noted in normal subjects at the end of 4, 24, 28 and 48 hours after the administration of aspirin and, later, in a similar study after the administration of citro-carbonate. The rise in Raymond's plasma, therefore, remains unexplained.

The 4 girls (table 3) started the study with a mean dietary intake of 44 mg of ascorbic acid per day. Their plasma levels seemed to be grouped into 2 pairs: Jo and Stasia showed plasma levels markedly above those of Betty Lou and Sharon. The variations in intake and in plasma ascorbic acid were small from day to day. After the beginning of the saturation period the increases in plasma ascorbic acid of all the girls were abrupt and of about the same degree. When apparent saturation levels had been attained, daily variations for all of the subjects were marked. This confirms the observations of Storvick and Hauck ('42), who reported wider variations in daily plasma values on high intakes than on low intakes

of ascorbic acid. Variations were especially marked for Jo, who claimed to have been ill [4] on the eleventh and twelfth days of the study.

Data for the first 2 days of the 80 mg period showed the same variation and heights as those during the saturation period, but for the last 5 days, variations were less marked. Sharon "leveled off" at about 0.9 mg %, which seems to be about 0.1 mg % lower than her saturation level. Stasia showed the greatest daily variation during the third period, with values ranging from 0.94 to 0.74 mg % during the last 5 days. Betty Lou dropped steadily from a near-peak of 1.35 to 1.13 mg %, a value lower than any since the beginning of her saturation values. Jo was the only one of the 4 girls whose data showed a definite upward climb after the first drop on the 80 mg level.

It will be observed that while the boys' intake for the first period is higher than the girls', there is an even greater difference in their plasma levels. This may be due in part to the difference in developmental age between the 2 groups, or the low values for the girls may reflect subsistence on a diet lower in ascorbic acid for a considerable time before the study began.

Peak values during saturation were about the same for both groups, but the boys attained their peaks somewhat before the girls, and their values dropped more abruptly when the intake was reduced.

While it would have been interesting to know whether the children, and especially Betty Lou, could maintain for prolonged periods these high plasma levels on the intake recommended by the National Research Council ('45), the results for this week suggest that their plasma values would be maintained above the level of adequacy according to Butler ('40).

DISCUSSION

In attempting to evaluate the results of this study, the findings of other research workers need to be reviewed. Investi-

[4] This subject was somewhat unstable. The nurse reported no evidence of illness.

gators have used various approaches to the problem of determining the ascorbic acid requirement of man. Some of the studies are based on plasma ascorbic acid levels, others on urinary excretion levels, and still others combine and compare the two. In order to study requirement, the daily intake of ascorbic acid must be known.

Some investigators have questioned the existence of any relationship between ascorbic acid requirement and body weight (Hathaway and Meyer, '41; Ralli et al., '39). Nevertheless, many workers have reported their results in milligrams per kilogram of body weight. Of 30 adults whose requirements for tissue saturation were studied by Belser, Hauck and Storvick ('39); Ralli, Friedman and Sherry ('39); Todhunter and Robbins ('40); Storvick and Hauck ('42); Fincke and Landquist ('42); and Kline and Eheart ('44), the estimated requirements of 24 fell between 1.3 and 1.8 mg per kg. Bryan et al. ('41) concluded on the basis of a survey that there is a close relationship between ascorbic acid intake per kg of body weight and fasting plasma ascorbic acid values. In their experiment, intakes of 1.7 to 1.9 mg per kg resulted in high fasting plasma ascorbic acid values, typical of tissue saturation. Fincke and Landquist ('42) found that ascorbic acid intakes of 0.8 to 1.2 mg per kg were necessary to maintain fasting plasma ascorbic acid values of about 0.8 mg % in 4 adult subjects, and Dodds and MacLeod ('44) observed that 1 mg per kg was adequate to cause a gradual increase in plasma values for 12 subjects who had been moderately depleted before being given increasing levels of ascorbic acid.

The 31 mg intake found by Hathaway and Meyer ('41) to maintain tissue saturation in 4 preschool children, was equivalent to 1.5 to 1.8 mg per kg for these children. The 5 children from 7 to 12 years of age studied by Roberts and Roberts ('42), required from 1.7 to 2.4 mg per kg to maintain tissue saturation. Thus, on the basis of body weight, requirements for tissue saturation of these children and of adults do not appear to differ greatly, although individual variations are apparent.

In the present study, mean fasting plasma values of from 0.9 to 1.2 mg % were maintained by the 4 adolescent girls on the allowance recommended by the National Research Council ('45), which was equivalent to 1.3 to 1.7 mg per kg. The National Research Council allowance, which was equivalent to 1.9 to 2.1 mg per kg for the 4 boys, maintained mean fasting plasma values from 0.8 to 1.0 mg % during the last 5 days of the 1-week period on this intake (see table 3). For these young adolescents, the allowances of ascorbic acid recommended by the National Research Council ('45) resulted in fasting plasma ascorbic acid values below those individually attained when saturation doses were given, although for 1 week these allowances were adequate to maintain plasma values which are associated with "good" stores of ascorbic acid. This first study on the ascorbic acid requirements of young adolescent boys and girls suggests that they may require somewhat more ascorbic acid per kilogram of body weight to maintain tissue saturation than do younger children or adults.

CONCLUSIONS

1. The daily allowance of ascorbic acid recommended for adolescents by the National Research Council ('45), as tested during this study, resulted for each subject in plasma values below those attained during the saturation period, but well above those considered adequate by Butler ('40). It would probably be advisable during the strain of adolescent growth to maintain all children near saturation, and since the recommended allowance accomplishes this in these subjects, diets may well be planned to include this amount for every child.

2. A mean plasma ascorbic acid concentration of 0.56 mg % was obtained from the survey of 81 children between 11 and 18 years of age residing at the Children's Farm Home. This value is about that (0.60 mg %) considered adequate by Butler.

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A STUDY OF SEX DIFFERENCES IN THE COMPOSITION OF RATS, WITH EMPHASIS ON THE LIPID COMPONENT

SEX DIFFERENCE IN SUSCEPTIBILITY TO ESSENTIAL FATTY ACID DEFICIENCY WITH HIGH AND LOW FAT DIETS [1]

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ONE FIGURE

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In the course of an investigation of the influence of essential fatty acids on various fat fractions of the albino rat, data were obtained on the composition of the experimental animals in terms of total lipid, non-lipid solids, and water content. These findings augment the limited data in the literature, particularly with regard to the comparative fat storage of male and female animals.

Boycott and Damant ('08) in a study of the fat content of male and female rats, guinea pigs, and mice stated that females tend to have a higher fat content. For rats, 41 males and 42 females raised on the ordinary laboratory diet, the detailed composition of which is not given, they obtained average values for the total fatty acids of 4.4 and 5.6%, respectively.

[1] Taken from a thesis presented to the Graduate Faculty of the University of Minnesota (1940) in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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Bachmann and coworkers ('38), reporting on the body constituents of albino rats on sugar diets, found no appreciable difference in fat content of males and females.

The data presented below constitute evidence for a sex difference in fat storage only when a specific dietary regimen is employed.

EXPERIMENTAL

At weaning time albino rats were given diet 551-B containing 84% sucrose, 12% crude casein, 4% salts,[4] and the necessary supplements which included 0.7 gm of dehydrated yeast [5] daily and adequate amounts of vitamin D,[6] carotene, and vitamin E (concentrate from wheat germ oil). This diet was continued for 4 weeks in order to deplete the fat reserves. During the following 8 weeks, which comprised the experimental period, the animals (litter mates) were placed on diets 560-B (60% sucrose, 20% lard, 15% casein, 5% salts [4]), 550-B (84% sucrose, 12% casein, 4% salts [4]), and diet 580-B which contained 71.7% hydrogenated coconut oil [7], 22.9% casein, and 6% salts.[4] In these diets purified casein was employed and constituted 12.5% of the total calories in each case. Six groups of rats were used, 3 male and 3 female, and each group contained 8 individuals. At the end of the experimental period the males of the control group (560-B) attained an average weight of 200 gm and the females 170 gm. Rats receiving the low-fat diet (550-B) attained weights of 170 and 150 gm for males and females, respectively. Those consuming the high-fat diet (580-B) attained weights of 108 and 104 gm for males and females, respectively. The growth curves are given in figure 1.

A second series of experiments was performed on rats prepared in the same way. For this purpose 6 males and 6 females were placed on diet 580-B, and 3 of each sex were maintained on diets 550-B and 560-B, respectively. Data were obtained

[4] Salt mixture 185 (McCollum and Simmonds, '18).

[5] Northwestern.

[6] Viosterol.

[7] Iodine value, 0.22. We are indebted to the research laboratories of Proctor and Gamble for this material.

on food and water consumption and fecal fat excretion, and the method for determining body fat was modified as described below. Since a different method of analysis was employed in this series, the data are reported separately in the tables, this phase of the investigation being designated as Series II and the one mentioned above as Series I.

At the end of the experimental period (the fifteenth week of life) the rats of Series I were killed by etherization after

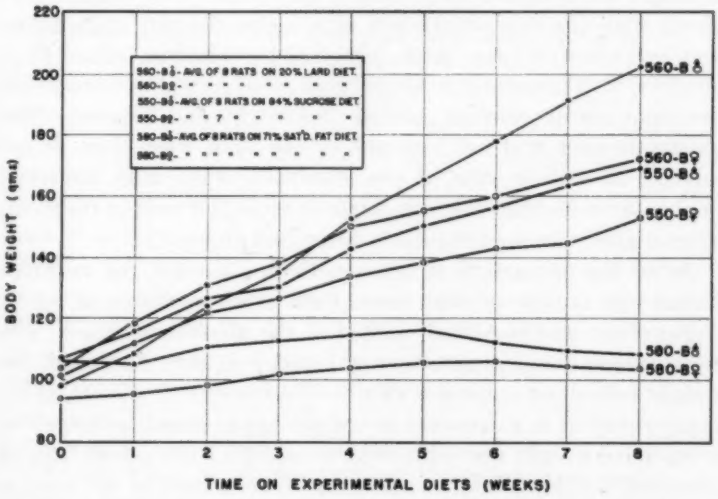

Fig. 1 Growth curves. Zero time represents the beginning of the eighth week of life. The first 3 weeks constitute the weaning period and the subsequent 4 weeks the period of fat depletion.

a 24-hour fast. The large intestine was discarded and the stomach and intestinal contents washed out with normal saline. The extraction followed, essentially, the procedure employed by Bloor ('26) in the extraction of beef heart muscle. The whole animal was comminuted in a meat chopper with fine cutter and dehydrated with 3 volumes of aldehyde-free alcohol (Dunlap, '06) for 1 to 2 hours. This aqueous alcohol solution was then decanted through a fluted filter paper and concen-

trated under carbon dioxide with reduced pressure to approximately 50 ml. It was then protected with carbon dioxide and set aside for petroleum ether extraction along with the main extract. The dehydrated tissue was extracted 3 times with 2 volume portions of boiling 95% alcohol under an atmosphere of carbon dioxide for 1, 1 and 0.5 hours, respectively. The combined extracts were concentrated under reduced pressure, with a stream of carbon dioxide bubbling through the solution, to a volume of 75 ml. This concentrate along with the one mentioned above was diluted with water and extracted 3 times with redistilled petroleum ether (b.p. 30–60°C.). The combined petroleum ether extracts were dried over anhydrous sodium sulfate overnight and filtered. The iodine number and total weight of the lipid were then determined. Weanling rats, 3 per analysis, were also analyzed in the same manner for comparison with the values obtained after maintenance on the diets described above.

After the extraction of lipid with hot alcohol the residual tissue was rinsed several times with small portions of anhydrous ether and the latter added to the alcohol solution. The residue was dried in air overnight and weighed. Although the weight remained constant, drying the residue at 105°C. overnight resulted in a decrease in weight approximating 7%. The oven-dried weight was employed to calculate the percentage of non-lipid solids. Water content was determined by difference.

In the second series of experiments the animals were treated as follows: After anesthetization the abdominal wall was opened and the blood withdrawn from the abdominal aorta for use in a study published elsewhere (Loeb, '42). The rats were then wrapped in paraffin paper and stored at —20°C. for approximately 2 months before analysis for carcass fat. The head, tail, and limbs were removed and the carcass digested for 4 hours in a 28% solution of potassium hydroxide made up with 50% alcohol. After cooling, the clear solution which contained a small quantity of insoluble material was carefully transferred through glass wool and made up to a known volume with 50% alcohol. The lipid content in terms of total

fatty acids plus unsaponifiable matter was determined in duplicate as follows: an aliquot portion was introduced into a large test tube and acidified with a hydrochloric acid solution of such strength as to leave the final alcohol concentration at 33%, by volume. After cooling, petroleum ether was added and the mixture well agitated. Centrifugation for 5 to 8 minutes gave a clean separation of layers and the petroleum ether layer was siphoned off. The extraction was repeated twice

TABLE 1

Iodine numbers of total lipids from whole animal (series I) and total fatty acids from carcass (series II).

GROUP	FAT IN DIET	SERIES I		SERIES II	
		No. of rats	Mean [1] iodine no.	No. of rats	Mean [2] iodine no.
Weanling rats		7 [3]	67.4 ± .35 [4]		
560-B ♂	20% lard	8	70.8 ± .62	2	76.9 ± .18
560-B ♀		8	68.9 ± .70	3	76.4 ± .18
550-B ♂	fat-free	7	61.6 ± .42	3	64.7 ± .30
550-B ♀		7	63.3 ± .45	3	65.0 ± .30
580-B ♂	71% hydrog.	8	45.5 ± 1.91	6	38.9 ± .41
580-B ♀	coconut oil	8	40.7 ± 1.27	6	39.0 ± .63

[1] Rosenmund-Kuhnhenn Method ('23).

[2] Wijs-Mercuric Acetate Method (Hoffman and Green, '39).

[3] This figure refers to the number of samples; 3 weanling rats per sample.

[4] Standard error of the mean calculated from the formula $\frac{S.D.}{\sqrt{N}}$ where S.D. represents the standard deviation, obtained from the expression $\sqrt{\frac{\Sigma x^2}{N}}$, in which x is the deviation from the mean.

more and after removing the solvent from the combined extracts the residue was dried in a vacuum oven at 60°C. for 1 hour, cooled in a desiccator and weighed. All of the duplicates checked well within 1%. For the determination of iodine numbers only the free fatty acids were used. Water was added to an aliquot portion of the original soap solution to reduce the alcohol concentration to 33%, by volume. The unsaponifiable fraction was extracted 3 times with petroleum ether and the remaining solution acidified and extracted as above.

Table 1 is a compilation of the mean iodine numbers of all the groups that were studied. Since different iodine number methods were employed in Series I and Series II, the 2 series are not strictly comparable to each other. However, the sex differences in unsaturation observed in Series I, if significant, are most likely due to the non-fatty acid portion of the total lipid. In Series II there is no suggestion of a sex difference in unsaturation of the total fatty acids of carcass.

Table 2 includes the mean values for the total composition of individuals studied in Series I. When analyzed statistically only group 580-B showed a significant sex difference in the storage of fat. The probability that this finding was due to chance was 1 in 59 ($P = 0.017$, Treloar, '42). There was no evidence of a sex difference in fat storage in groups 550-B and 560-B.

No difference in the percentage of non-lipid solids was observed between any of the groups regardless of sex or diet. Differences in water content due to sex could be shown statistically for groups 550-B and 580-B, those individuals with the higher fat content having also a lower water content. However, the probability was sufficiently close to the borderline for validity to warrant some reservation.

TABLE 2

Percentage composition of whole animals (series I).[1]

GROUP	DIET	SERIES I					
		No. of rats	Mean body weight	Water	Non-lipid solids	Total lipid	Total lipid
			gm	%	%	%	*gm*
Weanling rats		6[2]	127.4 ± 2.93	70.0 ± .81	24.7 ± 1.01	5.23 ± .02	6.66 ± .27
560-B ♂	20% lard	8	190.3 ± 6.31	67.0 ± .75	24.2 ± .47	8.85 ± .45	16.85 ± 1.24
560-B ♀		8	159.7 ± 2.55	65.5 ± .80	24.5 ± .42	10.01 ± .52	15.98 ± .92
550-B ♂	fat-free	8	154.5 ± 3.97	66.7 ± .55	25.0 ± .34	8.38 ± .59	12.95 ± 1.12
550-B ♀		7	131.9 ± 3.66	64.9 ± .38	25.8 ± .61	9.28 ± .56	12.24 ± .84
580-B ♂	71% hydrog.	8	99.2 ± 4.90	69.7 ± .68	24.9 ± .39	5.63 ± .69	5.58 ± .86
580-B ♀	coconut oil	8	95.2 ± 3.38	67.3 ± .74	24.7 ± .38	8.06 ± .54	7.67 ± .66

[1] For calculation of standard error of the mean see footnote in table 1.

[2] This figure refers to the number of samples; 3 weanling rats per sample.

Burr and Beber ('34) reported that rats on diet 550-B showed a higher metabolic rate, higher R.Q., and higher specific dynamic action of food than normal rats. In 1937, in a very carefully controlled series of experiments these same authors showed a marked difference in metabolic rate between rats maintained on diet 550-B and their controls which had been cured by administration of a curative oil. The difference between fat-deficient rats and animals from the stock colony receiving a sugar diet was still more striking. Since individuals

TABLE 3

Fat excretion and food and water consumption (series II).[1]

GROUP	NO. OF RATS	DAILY FAT EXCRETION	DAILY FOOD CONSUMPTION		DAILY WATER INTAKE
		gm	*gm*	*cal.*	*ml*
560-B ♂	3	0.035	7.19	34.5	8.7
560-B ♀	3	0.028	6.36	30.6	11.3
550-B ♂	3	0.014	6.76	26.0	11.1
550-B ♀	3	0.019	6.34	24.4	11.5
580-B ♂	6	0.028	3.83	28.0	8.5
580-B ♀	6	0.026	3.70	27.1	9.0

[1] These data were obtained on 1 or 2 groups of rats (3 per group), as indicated, during 6 weeks of the experimental period.

on diet 580-B consume as many calories as those on the fat-deficient diet (550-B)[8] (table 3) while at the same time undergoing scarcely any growth (fig. 1), and are losing no appreciable quantity of fat through the gut, it is reasonable to assume that their metabolic rate is even higher than that of rats on the fat-deficient diet. In addition, the greater fat content of females in group 580-B may be a reflection of a lower metabolic rate than that characterizing the males. It would appear from these observations that a deficiency of essential fatty acids has less effect on fat storage and metabolic rate in females than in males when both are maintained on high-fat diets.

[8] Burr and Burr ('30) showed that fat-deficient rats consume as much food as their cured controls.

DISCUSSION

The study of Deuel and coworkers ('44) of the comparative nutritive value of fats, using rats as experimental animals, has a bearing on the data reported above. They determined the composition of rats in terms of water, protein, lipid, carbohydrate, ash and calcium and concluded from their results that males have a higher water and protein content and a lower lipid, ash and calcium content. It is of interest to compare their results with the data we obtained on our control group, 560-B, which received a diet containing 20% lard (their animals consumed a diet containing approximately 30% fat). However, it should be noted that our rats were subsisting on a diet very low in fat for 4 weeks after weaning while theirs were placed on a 30% fat diet immediately after weaning. This probably accounts for the higher lipid values obtained by Deuel et al.

The values for protein, carbohydrate and ash obtained by Deuel and collaborators, when totalled, agree closely with our values for non-lipid solids, namely, 23.7% for males and 23.2% for females as against our values of 24.2% and 24.5% for males and females, respectively. We were not concerned with the carbohydrate and ash content. As for the total non-lipid solids, our difference between the means (0.3), was evidently not significant with a standard deviation ($\sqrt{\Sigma x^2/N}$) of 1.32 for the males and 1.20 for the females (8 rats in each group). In view of the large number of rats in the groups compared by Deuel et al. it would appear that the difference due to sex in protein as well as ash content is, in fact, a true statistical difference. It is well to note that these differences are obscured when total non-lipid solids are considered alone since the increased ash content found in females tends to mask their decreased protein content.

The sex difference in water content in our group 560-B (20% lard) was of the same magnitude as that observed by Deuel et al. but we are unable to establish statistical validity for this difference. The "t" value (Fisher, '38) was 1.3 whereas only a value in excess of 2.2 (the 5% level; i.e., 5

chances in 100 that the difference could be due to random sampling) could be accepted as being indicative of a valid difference. As mentioned above, the sex difference in water content for groups 550-B (fat-free diet) and 580-B (high saturated fat diet) was significant having "t" values of 2.3 and 2.2, respectively.

As for the lipid content our difference due to sex in group 560-B was of the same order of magnitude as that observed by Deuel et al. but, again, we could not establish statistical validity, the "t" value being 1.6. For group 550-B the "t" value was 1.1 while group 580-B showed a definite difference with a "t" value of 2.7.

Under the conditions of our experiment there was no statistically valid sex difference in the fat content of normal animals. The difference observed in our high-fat group (580-B) may therefore be ascribed to the lack of essential fatty acids which affects the males more than the females. It may well be, as Sinclair ('40) has suggested, that the requirement for essential fatty acids is greater on a high-fat diet. In this case one could infer that essential fatty acids are important in fat storage, and that females, probably through more efficient utilization of original stores of essential fatty acids, have a more effective fat-storing capacity than males. Or, to express it differently, the female may possess a more favorable fat economy when subjected to a condition of essential fatty acid deficiency while at the same time deriving most of its energy from fat. This is, essentially, an extension of an observation reported by Burr and Burr ('29) on rats consuming a fat-free diet (550-B). At the time of the growth plateau they found that males attain a weight equal to 70% of their controls receiving lard, while the weight of females is 80% of their controls. Furthermore, the eventual decline in weight proceeds more rapidly in males.

An examination of the iodine numbers in table 1 and the total lipid values in table 2 leads to the following conclusions: 20% lard in the diet contributes enough linoleic acid to maintain a normally high iodine number in the deposited fat; on

a fat-free, high carbohydrate diet much fat of low iodine number is synthesized and deposited; on a diet very rich in saturated fat and deficient in linoleic acid fat storage is less impaired in females than in males and there is a ready exchange of fatty acids in the tissues which results in very low iodine numbers. This supports the conclusion reached by metabolic studies that a severe deficiency of essential fatty acids does not prevent fat synthesis (Burr and Beber, '37) or mobilization of ingested fatty acids (Barnes, Rusoff and Burr, '42).

SUMMARY

1. Male and female albino rats were raised on 3 simplified diets; the control diet contained 20% lard while the other 2, deficient in essential fatty acids, were fat-free and rich in saturated fat (hydrogenated coconut oil), respectively. The composition of these individuals in terms of total lipid, total non-lipid solids, and water content was determined in addition to the unsaturation of body fat.

2. A sex difference in the unsaturation of fatty acids obtained from the body fat of rats raised on the 3 respective diets could not be established.

3. Evidence was presented showing that on a high-fat diet deficient in essential fatty acids females store more fat than males, and it was adduced that the latter are more sensitive to a deficiency in essential fatty acids than the females when both derive the bulk of their calories from fat.

4. Rats receiving a diet very high in saturated fat, but lacking essential fatty acids, are unable to effect an appreciable increase in the amount of total body lipid, but they readily exchange the dietary fatty acids with those in the tissues.

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AMINO ACID CONTENT OF FEEDS

I. LEUCINE, VALINE, ISOLEUCINE AND PHENYLALANINE [1]

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The rapid development of microbiological methods for amino acid determinations has greatly facilitated their application to the estimation of amino acids in natural materials. Considerable data are now available to show that these techniques can be utilized to determine the amino acid composition of natural materials following hydrolysis without preliminary removal of fat, water or carbohydrate material. The importance of such data for the judicious selection of the protein components of the diet to assure adequacy of the essential amino acids is well recognized. The present study was initiated to obtain information on the amino acid content of the more common feeds, particularly those used in poultry feeding. The values for valine, isoleucine, phenylalanine and leucine are presented in this paper.

EXPERIMENTAL

The feed samples were obtained by random sampling of the supplies of commercial feeds available. For some of the feeds

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(corn, corn gluten meal, meat and bone scraps, soybean oil meal, alfalfa leaf meal, and milo) additional samples were obtained from different sources. All samples were finely ground prior to assay to assure homogeneity.

The techniques used for the valine, leucine and isoleucine determinations were described previously (Schweigert et al., '44, '45). *L. arabinosus* was used as the test organism for these determinations. The determinations of phenylalanine were carried out by the use of *L. arabinosus* and *Leuconostoc mesenteroides* P-60. The medium used for the phenylalanine assay with *L. arabinosus* was the same as that used previously with the appropriate amino acid omitted from the basal medium. In the present studies *L. casei* factor and pyridoxamine were also included in the basal medium for *L. arabinosus* at levels of 0.02 and 1 μg per 10 ml of culture medium, respectively.

The medium used with *Leuconostoc mesenteroides* P-60 was the same as that used in earlier work (Riesen et al., '46) which is a modification of the medium described by Dunn and associates ('44). This organism was used for comparative tests, since the recent findings of Lyman ('46) and Lyman et al. ('46) show that *L. arabinosus* has the capacity to synthesize phenylalanine under certain test conditions. However, in the present work, excellent agreement was obtained with the use of both organisms for the determination of phenylalanine.

All amino acid standards used were critically tested for their purity to eliminate this factor as a possible source of error by comparing them with analytically pure standards. The amino acid values obtained are expressed on the basis of 100% activity for the l-isomer and no activity for the d-isomer.

The samples were hydrolyzed by autoclaving with 2N HCl at 15 lbs. pressure for 10 hours. This procedure has been found satisfactory for maximum liberation of amino acids (Schweigert et al., '44, '45; Riesen et al., '46). After hydrolysis, the samples were neutralized and aliquots taken for assay.

The results are expressed both as the per cent of each amino acid in the sample as analyzed and as the per cent in the crude

protein (calculated to 16% nitrogen). The determinations were made in duplicate or triplicate and all values were checked to within ± 5%. For comparison, results are also included for 3 purified proteins: casein, egg albumin and gelatin. The data are summarized in table 1.

To obtain further information on the usefulness of these individual values for calculating the amino acid content of rations composed of several sources of protein, 2 rations were analyzed for their amino acid content and the values compared with the calculated figures. These rations contained corn, oats, soybean oil meal, dried skim milk, corn gluten meal, fish meal, alfalfa leaf meal, meat and bone scraps and bone meal as constituents which supplied protein. The calculations were based on the analytical figures taken from the lots of feed used in preparation of the rations. The values found by calculation and determination are shown in table 2.

RESULTS AND DISCUSSION

The data presented in table 1 show that considerable variation exists in the amino acid content of feeds both when compared on the undried basis and on a protein basis. Large amounts of leucine were found in corn, corn gluten meal, milo and blood meal proteins. Isoleucine, however, is very low in blood meal. This is to be expected since several workers (Block and Bolling, '43; Brand and Grantham, '46; Devlin and Zittle, '44; and Orten et al., '45) have observed by analysis and in feeding tests that the principle protein, globin, is markedly deficient in this amino acid. The rice products tended to be low in all 4 amino acids.

The amino acid values obtained for different sources of the same feed were quite uniform for the corn products, milo, alfalfa leaf meal and soybean oil meal. Of the 4 amino acids tested, the values for phenylalanine were the most consistent, while those for leucine were the most variable. Rather divergent results were obtained for the various samples of meat and bone scraps. This is probably due to the varying composition of different lots of this feed. The amount of connective

TABLE 1

Amino acid content of feeds. All values are expressed as per cent of the undried sample and of the protein.

SAMPLE	PROTEIN (N × 6.25)	VALINE		ISOLEUCINE		PHENYLALANINE		LEUCINE	
		In the sample	In the protein	In the sample	In the protein	In the sample	In the protein	In the sample	In the protein
Corn	9.0	0.48	5.3	.38	4.2	0.40	4.4	1.04	11.6
Corn	9.06	0.47	5.2	.37	4.1	0.39	4.3	0.86	9.5
Corn	9.3	0.46	4.9	.38	4.1	0.39	4.2	0.89	9.6
Corn gluten meal	46.9	2.66	5.7	2.40	5.1	2.53	5.4	7.5	16.0
Corn gluten meal	49.0	2.78	5.7	2.43	5.0	2.57	5.2	7.6	15.5
Milo	11.6	0.57	4.9	0.51	4.4	0.47	4.1	1.38	11.9
Milo	12.4	0.55	4.4	0.51	4.1	0.48	3.9	1.14	9.2
Oats	11.6	0.66	5.7	0.52	4.5	0.52	4.5	0.74	6.4
Wheat	15.2	0.73	4.8	0.58	3.8	0.67	4.4	0.85	5.6
Wheat bran	16.0	0.82	5.1	0.54	3.4	0.53	3.3	0.83	5.2
Rice bran	16.0	0.70	4.4	0.50	3.1	0.47	2.9	0.69	4.3
Rice polish	15.6	0.59	3.8	0.40	2.6	0.40	2.6	0.57	3.7
Alfalfa leaf meal	18.6	1.10	5.9	0.96	5.2	0.79	4.2	1.28	6.9
Alfalfa leaf meal	18.5	1.04	5.6	0.93	5.0	0.75	4.1	1.17	6.3
Soybean oil meal	43.8	2.23	5.1	2.32	5.3	1.93	4.4	3.04	7.0
Soybean oil meal	44.8	2.32	5.2	2.14	4.8	2.04	4.5	3.16	7.1
Cottonseed meal	40.9	2.13	5.2	1.54	3.8	1.75	4.3	2.18	5.3
Linseed meal	42.7	2.15	5.0	2.2	5.2	1.83	4.3	2.13	5.0
Peanut meal	41.8	1.87	4.5	1.74	4.2	2.06	4.9	2.13	5.1
Cow peas, defatted	24.2	1.30	5.4	1.26	5.2	1.20	5.0	1.66	6.9
Fish meal	69.6	4.45	6.4	3.63	5.2	2.8	4.0	5.4	7.8
Meat and bone scraps	52.1	2.84	5.5	1.92	3.7	2.18	4.2	3.16	6.1
Meat and bone scraps	52.0	2.31	4.4	1.54	3.0	1.81	3.5	3.24	6.2
Meat and bone scraps	45.0	1.91	4.2	1.39	3.1	1.44	3.2	2.32	5.1
Blood meal	85.0	6.7	7.9	1.02	1.2	5.0	5.9	9.8	11.5
Sardine meal	65.1	4.08	6.3	3.27	5.0	2.57	3.9	4.69	7.2
Bone meal	33.1	1.06	3.2	0.65	1.96	0.76	2.3	1.29	3.9
Dried skim milk	35.4	2.8	7.9	1.97	5.6	1.58	4.5	3.42	9.7
Dried whey	10.6	0.68	6.4	0.66	6.2	0.35	3.3	0.99	9.3
Casein	95.7	6.7	7.0	5.9	6.2	5.2	5.4	9.35	9.8
Egg albumin	88.9	6.28	7.1	5.4	6.1	4.7	5.3	7.2	8.1
Gelatin	82.2	2.8	3.4	1.78	2.17	2.07	2.52	3.3	4.0

tissue, etc., in relation to the amount of muscle tissue would undoubtedly affect the amounts of the amino acids in the total protein. Furthermore, gelatin, derived from collagen and other connective tissue proteins, is rather low in these amino acids.

Some comparative values are available from the literature on the amino acid content of these feeds and are summarized by Block and Bolling ('45). The greatest discrepancies in the values are for valine and leucine. In general, the former were higher and the latter lower than those quoted by Block and Bolling. Results obtained microbiologically for some of these materials are published elsewhere (Kuiken et al., '43; Brand et al., '45; Stokes et al., '45; Hier et al., '45; McMahan and Snell, '44).

TABLE 2

Amino acid content of rations determined by actual analysis and calculated from the values obtained for the components.[1] *All values are expressed as per cent in the ration.*

RATION	LEUCINE	VALINE	ISOLEUCINE	PHENYLALANINE
No. 1 (determined)	2.49	1.17	1.06	1.07
No. 1 (calculated)	2.54	1.33	1.10	1.14
No. 2 (determined)	2.47	1.27	1.10	1.14
No. 2 (calculated)	2.64	1.42	1.21	1.19

[1] The amount of each amino acid contributed by the various protein ingredients was calculated on the basis of the percentage of that ingredient in the ration and from the data in table 1.

It is rather unlikely that a deficiency in leucine, valine, isoleucine or phenylalanine would be encountered with rations containing sufficient protein from a combination of feeds such as corn, oats, corn gluten meal, soybean oil meal, dried skim milk, fish meal, alfalfa leaf meal and meat and bone scraps. The leucine, valine, isoleucine and phenylalanine contents of poultry rations made up of these feeds were determined (table 2). The content is well above the estimated requirements for chickens, namely, 0.5% isoleucine, 1.5% leucine, 0.7% valine and phenylalanine, 0.5 to 1.5%, depending on the

level of tyrosine (Almquist and Grau, '44; Grau and Peterson, '46). No data, however, are available on the quantitative requirements of turkeys for these amino acids. The values obtained by calculating the amount of each amino acid furnished by the 6–8 protein components of the ration agree rather well with the values obtained when the entire ration was analyzed. The values for valine showed the greatest difference, although none of the determined and calculated values differed by more than $\pm$ 7%. Calculations based on these figures may not agree as well as observed in the present study when other sources of these feeds are incorporated in rations and such rations analyzed for their amino acid content.

When results become available on the quantitative requirements of all essential amino acids for various animals and on the amounts present in various feeds one may then interpret the adequacy of the diet in terms of the content of each amino acid. The important problem associated with availability of each amino acid can then be more easily investigated.

SUMMARY

1. The leucine, valine, isoleucine and phenylalanine contents of various feed materials have been determined by the use of microbiological methods.

2. Values for these amino acids in different lots of corn, corn gluten meal, alfalfa leaf meal, milo and soybean oil meal were quite uniform. More variability was observed between different samples of meat and bone scraps.

3. The differences between the amino acid content of 2 rations calculated from data on individual ingredients and determined by actual analysis were within $\pm$ 7%.

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THE ROLE OF B_6-DEFICIENCY IN THE TRYPTOPHANE-NIACIN RELATIONSHIPS IN RATS[1]

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In several species of animals, the course of tryptophane metabolism is affected by the vitamin B_6 content of the diet. When the pyridoxine intake is adequate kynurenic acid is the metabolite which appears in the urine of rabbits, dogs and rats (Berg, '34; Correll et al., '38; Homer, '15). In rats and dogs maintained on a pyridoxine-deficient diet the normal metabolic derivative is replaced by xanthurenic acid (Lepkovsky and Nielsen, '42). Kynurenin has been postulated as the single intermediate through which the 2 end-products of tryptophane metabolism are derived (Lepkovsky et al., '43).

Data in a previous note (Rosen et al., '46) from this laboratory showed that a marked rise in the excretion of nicotinic acid and of N^1-methylnicotinamide occurred when *dl-* or *l*-tryptophane was administered orally or subcutaneously to rats. Since the normal metabolism of tryptophane in rats yields N^1-methylnicotinamide in addition to kynurenic acid, it was thought desirable to study the effect of a pyridoxine-free diet on this relationship.

[1] The authors wish to thank Miss Nancy Anne Hunter and Miss Betty Jean Peck for helpful technical assistance.

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EXPERIMENTAL AND METHODS

Pyridoxine deficiency was induced in rats by feeding a ration of the following percentage composition: Vitamin-free casein,[4] 35; sucrose, 58; salts (Hubbell et al., '37), 4; cottonseed oil, 3. The ration was supplemented with 0.5 mg of thiamine, 1.0 mg of riboflavin, 2.5 mg of calcium pantothenate, 10 mg of inositol, 100 μg of vitamin K [5] and 100 mg of choline chloride per 100 gm. Each animal received 2 drops of cod liver oil twice weekly. A high protein (35% casein) diet was adopted because of the claim by Cerecedo and Foy ('44) that pyridoxine deficiency can be accelerated in rats by such diets.

A simple quantitative procedure for xanthurenic acid based on the formation of a green ferric salt in an alkaline medium (Lepkovsky et al., '43) was used for estimating this compound. To a volume (1–3 ml) of undiluted rats' urine in a graduated cylinder, adjusted to pH 8.1 with 1 ml of a saturated $NaHCO_3$ solution, 1 drop of 1.7% ferric ammonium sulfate is added. The contents are diluted to 10 ml with distilled water mixed by inversion, and poured into a calibrated Evelyn tube. A blank is prepared by diluting 1 ml of saturated $NaHCO_3$ and 1 drop ferric ammonium sulfate to 10 ml with distilled water. Readings were made in the Evelyn colorimeter using filter 620, set at 100 with the blank tube. Pure xanthurenic acid,[6] was used as a standard.

The N^1-methylnicotinamide was determined in a filtered sample of urine by the acetone-fluorometric method of Huff and Perlzweig ('47).

Six male rats (86–155 gm) maintained on the above diet for 30 days showed but slight acrodynia and complete cessation of growth. On the other hand the metabolic aspect of B_6 deficiency was definitely manifested by the excretion of appreciable amounts of xanthurenic acid in the urine after repeated doses of *dl*-tryptophane as shown in table 1. It can be seen from table 1 that as the pyridoxine deficiency became more

[4] Smaco.
[5] Synkavite.
[6] Kindly furnished by Sharp and Dohme, Inc.

TABLE 1

Twenty-four-hour excretion of N¹-methylnicotinamide and xanthurenic acid on pyridoxine-deficient diet after oral doses of tryptophane.

DAY	RAT 1		RAT 2		RAT 3		RAT 4		RAT 5		RAT 6		N'-ME. AVERAGE
	N'-Me.[1]	Xant.[1]	N'-Me.	Xant.	N'-Me.	Xant.	N'-Me.	Xant.	N'-Me.	Xant.	N'-Me.	Xant.	
	μg	*mg*	*μg*	*mg*	*μg*	*mg*	*μg*	*mg*	*μg*	*mg*	*μg*	*mg*	*μg*
						After 11 days on pyridoxine-deficient diet							
12	40		19		61		146		80		75		70
						After 100 mg d,l-tryptophane orally							
13	200	5.3	590	1.8	340	4.0	841	3.0	750	1.4	652	9.2	562
14	193	1.6	187	0.4	85	1.7	267	0.8	250	0.3	260	2.3	207
15	113	0	148	0	90	0	104	0	256	0	125	0	139
						After 100 mg d,l-tryptophane orally							
16	298	6.0	620	4.1	254	14.1	682	6.5	718	3.7	435	6.2	501
17	183	1.0	200	0.4	106	1.4	177	0.7	237	0.6	187	1.2	180
18	56	0	102	0	85	0	106	0	194	0	136	0	103
21	55		70		33		67		119		60		67
						After 100 mg d,l-tryptophane orally							
22	83	7.6	276	10.8	83	15.9	220	14.5	375	9.8	151	9.1	175
23	77	0.6	93	0.6	35	3.6	57	2.2	125	1.0	60	0.9	74
24	50		42		22		57		119		52		57
						After 100 mg d,l-tryptophane orally							
26	79	7.2	240	9.4	40	13.1	127	7.1	150	17.5	120	9.9	126
27	39	0.6	52	0.6	13	2.3	42	1.4	116	1.3	43	0.4	51
						After 5 mg pyridoxine HCl daily for 4 days and then 100 mg d,l-tryptophane orally							
36[2]	175	0	203	0	200	0	212	0	350	0	210	0	225
37	125		184		78		55		85		68		99

[1] *N'-Me.* denotes N¹-methylnicotinamide. *Xant.* denotes xanthurenic acid.

[2] On the twenty-ninth day 50 mg of N¹-methylnicotinamide was given orally to each rat (see text, p. 564).

acute, as measured by the increase in xanthurenic acid, there was a progressive decrease in the excretion of N^1-methylnicotinamide. Two control rats maintained for 35 days on the same diet but including 2 mg % pyridoxine excreted 3420 and 1690 μg N^1-methylnicotinamide in 48 hours after a dose of 100 mg *dl*-tryptophane. This is in contrast to the average value 177 μg found in the experimental rats after 26 days of pyridoxine depletion.

To check the possibility that the destruction of N^1-methylnicotinamide was not increased during the pyridoxine deficiency, each animal received 50 mg of N^1-methylnicotinamide by stomach tube. Approximately 16 to 28% was recovered in the urine in 48 hours, which indicates a normal rate of destruction of N^1-methylnicotinamide (Huff and Perlzweig, '46). In another series of pyridoxine-deficient rats, an oral dose of 25 mg of nicotinamide resulted in normal excretion of N^1-methylnicotinamide, indicating that the mechanism for methylating nicotinamide is not impaired in a vitamin B_6 deficient rat.

After 31 days on the pyridoxine-free diet, each rat received 5 mg of pyridoxine hydrochloride daily for 4 days before administration of 100 mg *dl*-tryptophane. The excretion of xanthurenic acid ceases abruptly after an oral dose of vitamin B_6, as was previously shown by Lepkovsky et al. ('43). But the disappearance of xanthurenic acid was accompanied by a relatively small rise in N^1-methylnicotinamide excretion after a dose of tryptophane, whereas in animals maintained on a complete diet, as the 2 control rats mentioned above, the usual response to a 100 mg dose of *dl*-tryptophane is the excretion of 1–3.5 mg per 48 hours.

For a period of 2 weeks after the initial 5 mg pyridoxine supplement each animal received 50 μg of B_6 daily. This resulted in an average weight increment of 24 gm per rat per week, indicating the overall remission of the pyridoxine deficiency. However, the low level of N^1-methylnicotinamide excretion after the test dose of tryptophane remained unaffected. During this period of B_6 supplementation the addi-

tion of biotin and folic acid or of brewers' yeast and Wilson liver (Fraction L) did not affect the results.

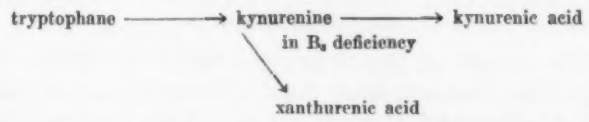
While the data given in this paper show the results on 6 rats only, for a period of 37 days, data involving experiments on 12 other rats, employing somewhat different diets and shorter periods of observation, yielded substantially the same results, namely, a significant decrease in N^1-methylnicotinamide excretion after a test dose of tryptophane. We have not found, to date, a single rat failing to exhibit this effect.

DISCUSSION

The data presented in this paper indicate that prolonged pyridoxine deficiency in half grown animals affects adversely the transformation of tryptophane to nicotinic acid in the rat. This metabolic defect is not rapidly restored after the animals ingest adequate amounts of the vitamin. That the hitherto observed abnormality in tryptophane metabolism involving the formation of xanthurenic acid is not directly related to the decrease of niacin formation is demonstrated by the simultaneous disappearance of xanthurenic acid from the urine.

The prolonged effect of B_6 deficiency on the synthesis of niacin and the failure to restore normal function even after 2 weeks of furnishing the vitamin point conceivably to changes in bacterial flora in the gut which are slow in developing. But the unlikelihood of the significant participation of the intestinal bacteria in the formation of niacin is emphasized by the extremely rapid response of rats to parenteral injections of tryptophane (Rosen et al., '46).

Furthermore, we attempted to test the relation of the



metabolic reaction (Lepkovsky et al., '43) to our problem. The administration of kynurenine, kynurenic acid and xanthurenic acid to rats on a complete diet failed to pro-

duce increased amounts of N^1-methylnicotinamide in the urine. This leads us to believe that the metabolic course from tryptophane to niacin in the rat is not via the above kynurenine pathway.

It was recently demonstrated that it is pyridoxal phosphate, rather than pyridoxine itself, which is involved in the enzymic systems concerned in transamination and decarboxylation of amino acids (Umbreit and Gunsalus, '45; Lichstein et al., '45) and in the synthesis of tryptophane (Umbreit et al., '46). Therefore, we thought it desirable to test the effect of administering pyridoxal upon the transformation of tryptophane to niacin in B_6-deficient rats. Subcutaneous and oral administration of 5 mg doses of pyridoxal daily proved as ineffective as pyridoxine itself in the experiments described above.

On the basis of our observations it is not possible at this time to propose a plausible hypothesis as to the possible mode of action of pyridoxine upon the tryptophane → niacin reaction. Until the enzyme systems involved in this transformation are more clearly defined this problem will probably remain unsolved.

SUMMARY

The omission of pyridoxine from the diet of rats results in a progressive decrease in the urinary excretion of N^1-methylnicotinamide after doses of tryptophane. This defect is only slightly overcome by the restoration of pyridoxine to the diet. The data indicate that the abnormal formation of xanthurenic acid in B_6 deficiency is not responsible for the impaired tryptophane to niacin transformation.

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THE EFFECT OF FAT LEVEL OF THE DIET ON GENERAL NUTRITION

I. GROWTH, REPRODUCTION AND PHYSICAL CAPACITY OF RATS RECEIVING DIETS CONTAINING VARIOUS LEVELS OF COTTONSEED OIL OR MARGARINE FAT AD LIBITUM [1]

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TWO FIGURES

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The small amount of information now available indicates that there may be an optimum fat level in the diet. The requirement of experimental animals for minimal amounts of certain unsaturated fatty acids is now well established (Burr, '42) although the single human experiment of Brown et al. ('38) was not conclusive. The suggestion that certain eczematous conditions in infants may result from fat deficiency has received some support (Hansen, '33; Finnerud et al., '41).

On the other hand, there is some evidence that excessive amounts of fat in the diet may be harmful. Higgins ('30) has reviewed the earlier medical literature, and reports reduced ability to work on high-fat diets. Loewy and coworkers ('42)

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observed increased erythrocyte destruction when large amounts of fat were added to the diet of dogs. Sako ('42) found a lowered resistance to pneumococci in mice which had received a high-fat diet (50%) for 6 weeks as compared with animals fed diets with less fat (23 or 5%).

Hoagland and Snider ('40) found greater gains in weight on diets containing 30 to 55% of fat, as compared with 5%; the fats used included several types of lard, oleo oil, and cottonseed oil. The efficiency of utilization, expressed as gain in weight per 100 cal. of food consumed, was greatest on the low-fat diets, however, indicating that the greater growth on the higher fat diets required a greater proportionate consumption of food. Hajdu ('42) studied the growth of working rats on diets in which fat and carbohydrate contents were varied reciprocally. The best growth, and the best work performance, were observed when the fat and carbohydrate contents of the diet were approximately equal while high-fat and low-fat diets resulted in poorer growth and performance. Crampton and Mills ('45) eliminated the effect of food consumption by feeding rats isocaloric amounts of diets containing 4% and 16% of fat. They report better growth on 4% than on 16%, whether the animals were fed ad libitum or isocalorically. Opposite results were obtained in a carefully planned experiment by Forbes and coworkers ('46a, '46b). They report greater growth with an increasing fat content of the diet at levels of 2, 5, 10 and 30% when isocaloric amounts were fed. The greatest improvement was seen between 2 and 5%. They attribute this effect to increased efficiency of utilization of the diet with a higher fat content.

The experiments to be reported here were designed to examine the question of optimum fat level under conditions of ad libitum feeding and isocaloric feeding at severely restricted levels of food intake. We have studied effects on growth, reproductive performance, physical capacity, nitrogen metabolism, blood composition, and body composition. The studies on nitrogen metabolism, blood composition, and physical capacity have been reported in part elsewhere (Miller et al.,

'46; Scheer et al., '47a). The present paper presents data on growth, reproduction and physical capacity on ad libitum feeding; the results of restricted feeding experiments will appear in succeeding papers (Scheer et al., '47b, '47c).

METHODS

The composition of the diets used is presented in table 1. The rations were based on those of Cerecedo and Vinson ('44).

TABLE 1

Percentage composition of diets used in fat studies.

DIETARY COMPONENT	DIET NUMBER								
	60a	60b	60c	61	62 and 67 [2]	63	64	65	66
Casein	25 [1]	25 [1]	25.5 [1]	27	28	31	37.5	34	39
Sucrose	67	67	65	61	54	40	12	26	0
Fat [3]	0	0	0	4	9	19	39	29	49
Cellulose [4]	3	3	3	3	3.5	4	4.5	4	4.5
Salt mixture [5]	4	4	4	4	4.5	5	6	5.5	6.5
Water-soluble vitamin mixture [6]	0.16	0.16	0.16	0.17	0.18	0.20	0.24	0.22	0.26
Fat-soluble vitamin mixture	1 [7]	1 [8]	1 [8]	1 [9]	1 [9]	1 [9]	1 [9]	1 [9]	1 [9]
Methyl linolate			1.5						
Fat, weight %	0	0	0	.5	10	20	40	30	50
Fat, % calories	(2.4)	(2.4)	(5.9)	11	21.5	39	64.5	51.4	69.6
Calories per gm	3.77	3.77	3.84	3.97	4.18	4.64	5.58	5.25	6.06

[1] Vitamin test casein from General Biochemicals, Inc., used. Commercial casein used in other diets.

[2] Diet 67 contains 7.2% of margarine fat and 2.8% cottonseed oil (including 1% in fat-soluble vitamin mixture).

[3] Cottonseed oil (Wesson oil) or margarine fat — the latter being designated by the addition of M to the diet numbers in the text.

[4] Cellu flour obtained from Chicago Dietetic Supply House.

[5] Osborne-Mendel salt mixture.

[6] The water-soluble vitamin mixture had the following percentage composition: choline, 93.50; thiamine chloride, riboflavin and pyridoxine, each 1.24; calcium pantothenate, 2.48; folic acid, 0.30.

[7] The fat-soluble vitamin mixture no. 1 had the following composition per 100 gm; α-tocopherol (Merck), 0.50 gm; carotene (General Biochemicals, Inc.), 20 mg; crystalline vitamin D_2 (Winthrop), 0.5 mg; ethyl laurate (Eastman) to 100 gm.

[8] Same as mixture no. 1 with methyl linolate replacing ethyl laurate.

[9] Same as mixture no. 1 with cottonseed oil replacing ethyl laurate.

Purified or synthetic materials were used because we desired to vary the fat content of the diet without changing the protein, vitamin or mineral intake. The composition of the diets was adjusted so that the ratio of protein, water-soluble vitamins, cellulose, and salts to calories would be the same in all diets. Thus, the intake of each of these components would be the same in isocaloric feeding, and proportional to caloric intake in ad libitum feeding. Ethyl laurate was used as a carrier of the fat-soluble vitamins in the fat-free diet (60a), methyl linolate was provided in diet 60b, and diet 60c was designed to provide a liberal allowance of methyl linolate for rats on restricted feeding. Cottonseed oil [2] was used as a vitamin carrier in all other diets. The margarine fat used in some of the diets contained no milk moisture or milk solids. The diets were mixed fresh at least once weekly in a mechanical dough-mixer. They were kept under refrigeration at all times. For comparison, a stock diet of natural foods was fed to 1 group of animals in some experiments. This diet was composed of ground whole wheat 34%, ground steel-cut oats 34%, skim milk powder 15%, cottonseed oil containing 1600 I.U. vitamin A and 160 I.U. vitamin D, 10%, alfalfa leaf meal 4%, dry yeast (Anheuser-Busch, strain G) 2%, sodium chloride 0.5% and calcium carbonate 0.5%.

Weanling rats from our stock colony were used. The animals were separated from the mothers at 21 days, and divided into experimental groups. Animals from any one litter were assigned to the several dietary groups in regular rotation. In the first experiment, approximately one-half of each litter was assigned to the ad libitum series, and the remainder to the restricted feeding series (Scheer et al., '47b). Each dietary group comprised 50 animals, 25 males and 25 females. The animals on ad libitum feeding were kept in groups of 4 in large cages. Five rats of each sex from each dietary series were placed in smaller individual cages for observations on food consumption. All animals were weighed once weekly.

[2] Wesson oil.

RESULTS

Growth

The growth curves for the males on cottonseed oil and margarine fat diets are plotted in figure 1 and those for the females in figure 2. The body weights fall into 3 rather definite groups in both cases. In the first experiment, the poorest growth was obtained with diets 60a and 60b, containing no

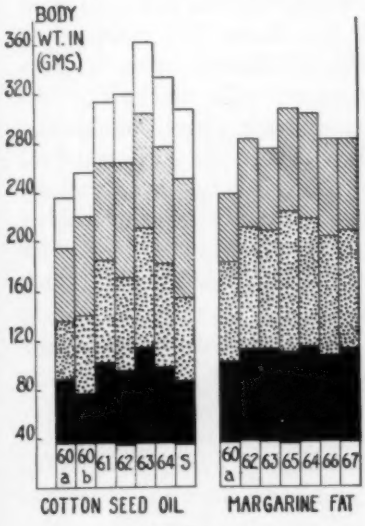

Figure 1

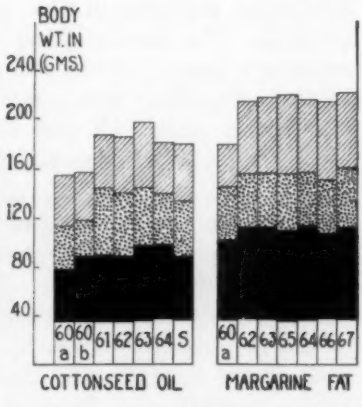

Figure 2

Fig. 1 The average body weight of weanling male rats at start (lower blank space), after 3 weeks (solid black), after 6 weeks (stippled), after 12 weeks (cross-lined) and after 18 weeks (top blank space). The figures in the lower blank space are diet numbers.

Fig. 2 The average body weight of weanling female rats at start (blank space), after 3 weeks (solid black), after 6 weeks (stippled), and after 12 weeks (cross-lined). The figures in the blank space are the diet numbers.

fat. Very much better growth was observed with diets 61, 62, and 64 containing 5, 10 and 40% fat, respectively. The best growth appeared on diet 63, with 20% fat. In the second experiment, growth was poor on 60a, much better on 67M,

62M (10%), 63M (20%) and 66M (50% fat), and best on 64M (40%) and 65M (30%). It is interesting to note that growth on 60a was much better in the second experiment than in the first, while growth on 62M and 64M was better than on 62 and 64, respectively, and on 63M was poorer than on 63. It does not seem possible to explain these latter differences without further experiments. Three conclusions are supported by the 2 experiments: (1) Growth is better on any diet containing fat than on 60a or 60b which had no fat. (2) The optimum fat content for growth lies above 10% and below 50%. (3) There are no consistent differences between diets containing cottonseed oil as compared with margarine fat. Statistical analysis of the data confirms these conclusions (table 2). The most striking differences in the cottonseed oil series are those between 60b and 61, and between 62 and 63. In the margarine series, 60a was decidedly inferior to the other diets while 64M and 65M were superior to diets with less fat. The growth curves for the female rats follow the same pattern (fig. 2 and table 2); diets 60a and 60b were inferior to the others in promoting growth. However, there were no significant differences among the diets containing 5 to 50% fat, as regards growth of females.

It is of interest to compare growth with food consumption on the various diets (table 3). Five rats of each sex were fed each of the experimental diets and were kept in individual cages; records were kept of food consumption. The gains in weight, and total calories consumed are presented in table 3 for the first 6 and 12 weeks of the experiment. In general, it appears that the greatest growth was accompanied by the greatest food consumption, although there are some exceptions among the females. It might accordingly be concluded that the differences in promoting growth in the diets used depend simply on variations in the amounts which rats will consume in ad libitum feeding. We have no positive proof that this is not the whole explanation but Forbes and coworkers ('46a, '46b) found similar differences with isocaloric feeding, and we have noted a superiority of diets containing fat in severely

TABLE 2

The mean weights of male and female rats after 12 weeks on diets containing different amounts of cottonseed oil or margarine.

Diet no.	% fat	MALE RATS Average weight [1]	M.D.: S.E.M.D.[2]	Diet no.	% fat	FEMALE RATS Average weight [1]	M.D.: S.E.M.D.[2]
			Cottonseed oil diet				
60a	0	193.7 ± 8.2	60b,2.4; 61,6.5; 62,4.7; 63, 10.5; 64,7.4; 8,5.9	60a	0	154.5 ± 4.3	61,6.2; 62,5.3; 63,7.6; 64,6.5; 8,4.7
60b	0	219.9 ± 5.9	61,4.9; 62,4.9; 63,5.0; 64, 5.8; 8,3.9	60b	0	157.1 ± 4.3	61,5.7; 62,5.0; 63,7.1; 64,6.0; 8,4.2
Stock	14	251.0 ± 5.2	63,6.3; 64,2.8	Stock	14	179.7 ± 3.3	63,3.5
61	5	264.1 ± 7.0	63,4.2	61	5	187.0 ± 2.9	63,2.2
62	10	264.3 ± 6.8	63,4.2	62	10	135.4 ± 3.9	63,2.2
64	40	277.6 ± 7.8	63,2.5	64	40	190.3 ± 3.5	
63	20	304.0 ± 5.6		63	20	197.0 ± 3.6	
			Margarine fat diet				
60a	0	238.2 ± 5.1	62,6.6; 63,5.4; 64,10.3; 65, 10.0; 66,6.4; 67,6.7	60a	0	179.4 ± 3.0	62,3.3; 63,3.4; 64,3.5; 65,5.1; 66,3.1; 67,4.9
63	20	274.6 ± 4.4	64,5.0; 65,5.1	66	50	193.5 ± 3.3	
66	50	282.1 ± 4.5	64,3.6; 65,3.8	62	10	194.0 ± 3.2	
62	10	282.5 ± 4.4	64,3.5; 65,3.8	64	40	194.8 ± 3.2	
67	10	282.7 ± 4.2	64,3.6; 65 3.8	63	20	197.0 ± 4.3	
64	40	303.0 ± 3.7		65	30	199.4 ± 2.5	
65	30	306.5 ± 4.5		67	10	201.0 ± 3.9	

[1] Including Standard Error of Mean calculated as follows $\sqrt{\Sigma d^2/n} / \sqrt{n}$ where "d" is the deviation from the mean and "n" is the number of observations.

[2] Ratio is given for diets in which growth is significantly greater. In each case the ratio follows the number of the dietary group compared. The figure 2.5 is taken as the lowest significant ratio (only 1 chance in 100 that this is not significant).

TABLE 3

The relation of average gain in weight to food consumption of rats (5 in each group) kept in individual cages for 12 weeks.

DIET NO.	MALE RATS						FEMALE RATS					
	6 weeks			12 weeks			6 weeks			12 weeks		
	Average gain	Total Cal.e	Gain/ 100 Cal.	Average gain	Total Cal.	Gain/ 100 Cal.	Average gain	Total Cal.	Gain/ 100 Cal.	Average gain	Total Cal.	Gain/ 100 Cal.
		gm			gm			gm			gm	
					Cottonseed oil diet							
60a	72.6	1310	5.54	113.2	3370	3.36	67.0	1320	5.07	98.6	3120	3.06
60b	30.8	880	3.50	139.4	2740	5.09	50.8	1090	4.66	112.2	2940	3.82
61	137.6	1720	8.00	220.4	4020	5.50	100.0	1720	5.80	140.2	3750	3.74
62	134.0	1920	6.97	226.8	4360	5.20	98.2	1600	6.14	138.6	3440	4.02
63	173.6	2260	7.66	250.2	4790	5.24	100.2	2020	4.96	164.6	3820	4.30
64	147.8	1865	7.91	215.0	4230	5.09	104.0	1880	5.54	138.0	3860	3.58
					Margarine fat diet							
60a	139.0	1680	8.27	189.6	3740	5.07	100.0	1420	7.04	134.6	3180	4.23
62	164.6	1780	9.25	241.2	3920	6.15	110.0	1610	6.83	148.0	3280	4.51
67	157.5	1760	8.95	234.4	3820	6.15	113.8	1430	7.95	154.0	3040	5.06
63	174.0	1890	9.20	239.0	4020	5.95	118.8	1740	6.82	167.2	3540	4.74
65	179.2	2000	8.96	267.4	4360	6.12	112.0	1750	6.40	150.4	3590	4.19
64	160.0	1630	9.80	243.4	3770	6.19	118.2	1630	7.26	153.8	3250	4.72
66	158.0	1745	9.06	245.6	3840	6.37	103.8	1680	6.16	142.8	3440	4.14

restricted isocaloric feeding (Scheer et al., '47b, '47c). In this connection, it should be noted that the efficiency of utilization of the diets, as expressed by the gain in weight per 100 cal. of food consumed, varies only slightly, and in no consistent fashion among the diets. There are instances where the efficiency is lowest when the growth is least, but this is not invariably the case. The efficiency is greater in the males than in the females and in general the highest efficiency obtains for the period of rapid growth (first 6 weeks). These data are in agreement with our earlier results (Deuel et al., '44).

Physical capacity

The methods used for the determination of physical capacity and some of the results obtained have been reported elsewhere (Scheer et al., '47a). The method depends on a measurement of the time required for a rat to become exhausted when swimming with a regularly increasing work load. The results for the present experiments are summarized in table 4. Statistical analysis of these data shows the

TABLE 4

The physical capacity of rats fed diets containing different amounts of fat ad libitum.

SEX	WEEKS ON DIET	DURATION OF SWIM IN SECONDS									
		60a 0%	60b 0%	61 5%	62 10%	63 20%	65 30%	64 40%	66 50%	Stock 14%	67 [1] 10%
Cottonseed oil as the dietary fat											
♀	6	512	580	802	777	1062		922		1025	
		(5)	(5)	(6)	(5)	(5)		(3)		(4)	
♂	6	805	692	1019	921	1223		1143		955	
		(5)	(5)	(5)	(5)	(5)		(5)		(4)	
♂	12	645	637	769	850	846		1068		1084	
		(20)	(19)	(19)	(17)	(19)		(15)		(19)	
Margarine fat as the dietary fat											
♂	12	485			806	1174	1065	876	1122		1218
		(23)			(10)	(8)	(25)	(4)	(10)		(8)

Figures in parentheses are number of tests.

[1] 7.8% margarine fat and 2.2% cottonseed oil.

following significant differences for the males (mean difference/standard error greater than 2.5). In the first series (cottonseed oil) tested after 6 weeks, diet 63 was superior to 60a and 60b, and 61 was superior to 60b. After 12 weeks physical capacity was greater on diets 62, 64 and the stock diet than on 60a or 60b, while the stock diet was also superior to 61 and 62. In the second experiment (margarine fat), capacity was greater on 62M, 63M, 65M, 66M than on 60a, and greater on 63M and 67M than on 62M. These data do not present a wholly consistent picture, but it is evident that, in all cases, physical capacity is low on diets containing no fat, as compared with diets containing 10 to 50% fat. It is of particular interest that the stock diet, which does not support growth as well as diets 62 and 63, is superior to diet 62, and probably to 63, in the development of physical capacity. It is likewise of interest that diet 67M, which differs from 62M only in the presence of a small amount of cottonseed oil in place of part of the margarine fat, and which supports almost exactly the same amount of growth, is apparently superior in providing for development of physical capacity. It is unfortunate that so few animals were tested in this group.

Reproductive performance

The data on reproduction are summarized in table 5. The females in the cottonseed oil series were checked regularly for sexual maturity, as indicated by opening of the vagina. The table shows that maturity was attained more rapidly on the diets (61–64 and stock) containing fat than on the fat-free diet. After 16 weeks on the experimental diets, 8–10 females from each dietary group were mated with males from the same group. The litters were reduced to 7 animals each at 3 days after birth. Fertility, as indicated by the percentage of matings which resulted in pregnancy and birth, was lowest on diet 60b, where no pregnancies developed, and relatively low on 60a, where only 2 litters were born. The animals born to mothers on diet 60a were smaller when weighed 3 days after birth, and remained smaller during the lactation period than

those born to mothers on diets containing fat; the fat-free diet evidently does not support adequate lactation. Considerable variation in fertility and per cent mortality of the young rats was evident among the animals receiving the diets containing fat, but there was no consistent relation to fat content of the diet. Breeding tests were not performed with the animals in the second (Margarine fat) series.

TABLE 5

Reproductive performance of rats fed experimental diets. Bred 16 weeks from beginning of experiment.

DIET	AGE OF FEMALES AT MATURITY (WEEKS)	NO. OF MATINGS	% OF MATINGS SUCCESSFUL	AVERAGE TIME, DAYS, FROM MATING TO BIRTH	AVERAGE NO. OF ANIMALS PER LITTER	AVERAGE WEIGHT OF			% MORTALITY BEFORE 21 DAYS
						Litter at 3 days	Individuals		
							at 3 days	at 21 days	
60a	8.1	9	22.2	36.0	6.0	36.5	6.1	17.3	0
60b	9.1	9	0						
61	7.0	8	62.5	25.6	8.0	67.6	8.5	36.9	10
62	6.5	10	90.0	26.8	6.5	55.0	7.6	27.3	49
63	6.4	9	55.6	26.2	6.2	51.0	8.3	33.1	13
64	6.3	10	70.0	30.7	8.0	58.0	7.9	32.4	30
Stock	6.6	10	90.0	31.0	7.8	66.7	7.6	30.7	23

DISCUSSION

Where we have noted significant differences in performance related to the fat content of the diet, these have all been in the same direction; animals receiving diets containing 5 to 50% fat grow more rapidly, reach a greater final weight, have a greater capacity for exhausting work, attain sexual maturity earlier, are more fertile, and raise larger young than wholly comparable animals fed diets containing no fat. This was not the result of fatty acid deficiency, or deficiency of fat-soluble vitamins; the diet (60b) with added methyl linolate gave just as poor results as the diet (60a) containing only ethyl laurate. Growth was better on diets containing intermediate amounts of fat (20–30%) than on similar diets with small (5–10%) or large (40–50%) amounts. The nature of the fat, as between

cottonseed oil and margarine fat (hydrogenated vegetable oils), had little consistent influence on the results.

These conclusions are in agreement with those reached by Hoagland and Snider ('40) and by Forbes et al. ('46a, '46b). At least 2 factors are apparently involved in the superiority of diets containing fat, although our data do not permit an evaluation of the relative importance of these factors, or an estimate of other possible influences. The first factor is the greater intake in terms of energy values of diets containing fats. This was noted by Hoagland and Snider ('40) and is evident in our results. A second component influence is the greater efficiency of utilization reported by Forbes et al. ('46a, '46b) with diets containing larger amounts of fat. They found that the energy cost of metabolizing diets providing constant protein intake decreased with increasing ratio of fat to carbohydrate. Our data, however, provide evidence of an optimum fat intake somewhere between 30 and 50% of the diet (50–70% of total cal.) at least as regards growth. There is no evidence that the low-fat or fat-free diets give more efficient food-utilization as indicated by Hoagland and Snider ('40). In fact, the lowest efficiency (gain in weight/100 cal.) was obtained on the fat-free diet in all cases.

It appears from the tests of reproductive performance and physical capacity that factors other than fat content as such may be important. Particularly noteworthy is the superior performance of the stock diet in supporting exhausting work, as compared with synthetic diets of approximately the same fat content. It appears desirable to examine this question further, with numbers of animals sufficiently great to offset the variability inherent in the determination.

We originally planned to observe the effects of our experimental diets on longevity. It has proved impossible thus far to do this, but the results of such a study would be of particular interest; the more rapid growth observed on diets containing fat might well be associated with a shortened life span. It seems also of value to note that growth remains the most sensitive criterion among those which we have used in esti-

mating the effects of these diets; whether it is the most valid is more questionable.

SUMMARY

Ad libitum feeding to rats of diets varying in fat content from 5 to 50% (10 to 70% of total cal.) results in better growth, greater physical capacity, and better reproductive and lactation performance in rats than does feeding of diets containing minimal amounts of fat. The difference is not influenced by presence of methyl linolate in the fat-free diet. Optimum growth was observed on diets containing 20–40% fat. The most pronounced differences were observed in males. The effects are in part, but not entirely, attributable to greater caloric intake on the diets containing fat.

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THE EFFECT OF FAT LEVEL OF THE DIET ON GENERAL NUTRITION

II. GROWTH, MORTALITY AND RECOVERY IN WEANLING RATS MAINTAINED ON RESTRICTED CALORIES [1]

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ONE FIGURE

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Interest in phenomena related to undernutrition has been intensified in recent years by widespread conditions of famine. The studies of Keys and coworkers ('46) with typical famine diets have contributed valuable information concerning the effects of starvation on man. We wished to examine in more detail the effects on rats of variations in the fat content of purified diets under severe caloric restriction.

These experiments to be reported here were carried on simultaneously with those reported in the preceding paper of this series (Deuel et al., '47). Litter mates were divided between the experiments, the same diets were fed, and the same measurements of growth, reproductive performance and physical capacity were used.

[1] The subject matter of this paper has been undertaken in cooperation with the Committee on Food Research of the Quartermaster Food and Container Institute for the Armed Forces. The opinions or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or indorsement of the War Department.

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METHODS

The diets were designed so that the ratio of protein, vitamins or minerals to calories was the same in all diets. Thus, when the caloric intake was held constant the only variation among animals on different diets was in the intake of fat and carbohydrate; the intake of vitamins, proteins and minerals remained constant.

The animals were kept singly in especially designed cages ($7 \times 8 \times 6$ inches) in a room at constant temperature of 78° during the period of caloric restriction. On transfer to ad libitum feeding, the animals were kept in groups of 4 in larger cages ($16 \times 10 \times 11$ inches).

The animals on restricted calories were fed the diets on alternate days, at the same hour each day, in weighed portions. It was realized that this practice, necessitated by shortage of personnel, would influence to some extent the results of the experiment. However, we considered that comparisons between groups receiving different diets would be valid, since all groups were being dealt with in the same way. The animals were weighed once weekly.

RESULTS

Caloric restriction at various levels

In our first experiment, we wished to establish the lowest level of caloric intake which would permit survival during 12 weeks in weanling rats. Male rats 21 and 28 days old were used. They were fed the stock diet at levels ranging from 13 to 35 Cal. per day for 12 weeks. The general results are presented in table 1. The most striking feature of the results is the fact that the final weight attained depends on the caloric intake per day, regardless of the initial weight or age of the animal. The relation is linear and may be expressed as

$$W = aC - K$$

where W is the final weight in gm and C the caloric intake per day. The constants in this case have the values, $a = 8.25$ and $K = 47$.

TABLE 1

Weight changes in male weanling rats fed stock diet at varying levels of caloric intake for 12 weeks.

CALORIES FED PER ANIMAL PER DAY	NUMBER OF ANIMALS	AVERAGE WEIGHT		INITIAL AGE
		Initial	Final	
		gm	*gm*	*days*
34.7	9	55.4	217	28
27.7	8	46.2	180	28
24.1	7	39.7	142	21
23.1	9	60.4	142	28
19.1	7	42.8	112	21
18.8	9	57.5	106	28
17.1	7	40.8	95.5	21
14.7	7	38.7	74.5	21
14.5	10	56.2	72.7	28
12.9	7	39.5	59.9	21

Caloric restriction with variable fat content in the diet

On the basis of this preliminary work, it was concluded that weanling rats 21 days of age could survive for 12 weeks on a diet providing 12 Cal. per day. Accordingly, 300 animals, litter mates of those used by Deuel et al. ('47), were distributed among dietary groups, 25 males and 25 females in each group. They were placed in individual cages when 21 days old, and fed the experimental diets (60c, 61, 62, 63, 64, and stock) in weighed portions to provide 24 Cal. every second day. A few of the animals failed to consume their food and died within a day or 2 after transfer to the experimental cages; these were replaced, but data from them were not included in the records reported here.

The gains in weight on the different diets are presented in table 2. The standard errors of the mean weights range from $\pm$ 1.0 to 1.5. The standard error of a difference between 2 means would then be about $\pm$ 2.0. There is accordingly about 1 chance in 100 that a difference in weight as great as 5 gm could arise by chance. If a difference of 5 gm is then taken as significant, we may say that diets containing fat permit

better growth on severely restricted calories than does diet 60b, lacking in fat but containing adequate amounts of linoleic acid and the fat-soluble vitamins. The increase in weight is, of course, very small as compared with growth on the same diets fed ad libitum (Deuel et al., '47).

The mortality figures in table 2 represent the number of animals which died after 2 or more weeks on the experimental diets. It is remarkable that the greatest number of deaths

TABLE 2

Total average gains in weight (gm) of rats fed experimental diets at a level of 12 cal. per day for 12 weeks after weaning.

DIET	% FAT	INITIAL WEIGHT	GAIN IN WEIGHT AFTER				NO. OF DEATHS
			3 weeks	6 weeks	9 weeks	12 weeks	
			Males				
60c	0[1]	36.4	3.2	7.1	10.1	18.5	4
61	5	33.9	6.1	13.3	14.9	25.1	8
62	10	36.1	5.9	10.8	12.0	19.7	5
63	20	35.8	7.1	16.6	15.7	20.0	4
64	40	36.6	6.7	14.6	16.7	25.6	3
Stock	14	35.4	5.6	12.9	13.7	21.1	2
			Females				
60c	0[1]	36.4	2.0	4.7	6.3	15.3	5
61	5	36.1	0.1	4.7	10.5	20.9	17
62	10	34.3	4.7	8.9	10.7	19.5	9
63	20	36.1	6.8	11.1	11.4	21.0	4
64	40	35.1	6.6	11.0	12.9	21.7	3
Stock	14	36.8	3.0	9.9	10.0	16.5	2

[1] Contains 2.5% methyl linolate.

occurred on diets 61 or 62, with less on diets 60c and 63, still less on 64, and the least on the stock diet. Most of the deaths occurred 3 to 4 weeks after the beginning of the experiment.

Cottonseed oil, the fat used in these diets, contains about 40% linoleic acid (Hilditch, '40). Diets 60c and 61 therefore have approximately the same content of linoleic acid, but in the former it is present as the methyl ester, and in the latter as glycerides with other fatty acids. It is difficult to account for the observed mortality in the basis of this difference

in composition. Animals fed these diets ad libitum showed no mortality.

It is possible that the observed mortality could have resulted from drafts in the animal room, since the animals on different diets were grouped in different racks of cages. However, the animals on 60c and 61 were in the same cage rack, and the room was small and ventilated by a forced air draft, so this explanation seems unlikely. Death in some cases was associated with pulmonary inflammation and hemorrhage from the nostrils. In other cases, these symptoms were absent.

Recovery from the period of caloric restriction

Weight. At the end of the 12-week period of restricted caloric intake, the animals were placed on ad libitum feeding on the same diets, except that diet 60c was replaced by 60b containing 1% methyl linolate instead of 2.5%. Record was kept of the weight of the males for 12 weeks, but females were weighed only for 3 weeks after transfer (fig. 1). After 3 weeks, breeding tests were started with most of the females, and the weights would consequently not have represented merely growth changes. In general, the variations in weight between animals on different diets were small, but statistically significant differences (difference/standard error >2.5) were apparent between diet 60b containing no fat but with the addition of methyl linolate, and diets 62 to 64 containing 10 to 40% fat. Growth on the stock diet, with 14% fat was also inferior in some cases to growth on diets 62 to 64, but this may perhaps in part be attributed to the lower protein content of the stock diet (protein 15% of stock diet, 25 to 37% in diets 60 to 64).

Moreover, rapid growth was still occurring during the 9- to 12-week period after ad libitum feeding was instituted in the rats receiving diets which contained 10% or more fat while it had practically stopped in the rats receiving the low-fat diets. The following increments of growth were noted for the 9- to 12-week period: 60b, 4.3 gm; 61, 6.6 gm; 62, 17.3 gm; 63, 14.6 gm; 64, 27.1 gm and stock diet, 26.0 gm.

Maturity of females. Regular observations were made to determine the time of opening of the vagina in the female rats as a criterion of sexual maturity. None of the females became mature during the 12-week period of restricted feeding; controls fed the same diets ad libitum matured within 3 to 6 weeks after weaning (Deuel et al., '47). However, the vagina opened very shortly after transfer from restricted to ad libitum feeding. Maturity was evident earlier in rats fed the diets containing fat, as compared with the fat-free diet (table 3).

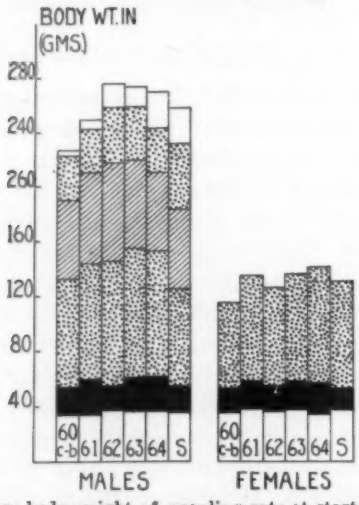

Fig. 1 The average body weight of weanling rats at start (lower blank space), after 12 weeks on restricted calories (solid black), after 3 weeks ad libitum (stippled), after 6 weeks ad libitum (lined), after 9 weeks ad libitum (upper stippled), and after 12 weeks (upper blank space). The figures in the lower blank space are the diet numbers.

TABLE 3

Time (weeks) from transfer to ad libitum feeding to sexual maturity (opening of vagina) in female rats fed restricted amounts of diets varying in fat content for 12 weeks after weaning.

DIET	60b	61	62	63	64	STOCK
Fat content in %	0	5	10	20	40	14
Time to maturity, in weeks	2.1	1.7	1.5	1.1	1.4	1.2

TABLE 4

Reproductive performance of female rats recovering from a period of undernutrition.

DIET	FAT CONTENT	MATED WITH MALES FROM	NO. OF MATINGS	% OF MATINGS SUCCESSFUL	AVERAGE TIME FROM BIRTH TO MATING	AVERAGE NUMBER OF ANIMALS PER LITTER	AVERAGE LITTER WEIGHT AT		WEIGHT OF INDIVIDUALS AT		MORTALITY BEFORE 21 DAYS
							3 days	21 days	3 days	21 days	
	%				*days*		*gm*	*gm*	*gm*	*gm*	%
60b	0 [1]	60b	6	67	28	5.3					
							5.3	47.6	152	8.9	26.1	9
		N [2]	2	100	24	5.5					
61	5	61	3	100	25	8.3					
							6.8	47.7	219	7.1	32.4	0
		N	1	100	29	2.0					
62	10	62	4	100	31	8.3					
							7.7	64.3	167	8.4	27.8	35
		N	2	100	25	7.5					
Stock	14	Stock	5	60	34	6.0					
							7.0	53.8	175	7.7	23.3	14
		N	2	100	24	8.5					
63	20	63	5	100	26	7.6					
							8.5	60.8	230	7.2	31.8	12
		N	5	80	24	9.5					
64	40	64	5	80	30	9.5					
							8.9	72.3	283	8.1	30.6	8
		N	5	80	28	7.8					

[1] Contains 1% methyl linolate.

[2] N signifies males from stock colony.

Fertility and lactation. Breeding tests were initiated 5 to 6 weeks after transfer to ad libitum feeding. Five females from each dietary group were mated with males from the same group; litter mates were not mated. Five females from each group were also mated with males from the stock colony. In some instances, not enough females were available, and the number of matings was less than 10. The litters were not reduced in size after birth; they were weighed at 3 and 21 days. By allowing the female to keep the full litter, we felt that this would offer a more critical test of the lactation capacity than in the first experiment (Deuel et al., '47) where the litters were reduced to 7. A mating was considered unsuccessful if pregnancy had not developed within 2 months. The results of these tests are summarized in table 4.

Fertility was not greatly reduced except on the stock diet, and on diet 60b. Here only 3 out of 5, and 4 out of 6 matings, respectively, were successful when males from the same dietary group were used. When males from the stock colony (N) were used in 2 matings in each case, both were successful. It is regrettable that we were unable to make more matings with stock colony males, since it appears that the lowered fertility in these cases may be due to the males. The length of the period from the time at which the rats were placed together in the cage to the birth of the litter shows no consistent variations, except that the period was in general shorter when stock colony males were used.

The number of animals per litter, and the weight of the litter at 3 and 21 days, shows an increase with increasing fat content of the diet. The greatest difference is that between diets 60b and 61; diets 63 and 64 appear to be superior to the others in supporting lactation.

DISCUSSION

The results of this study are in agreement with other work from this laboratory: the work capacity of rats (Scheer et al.,

'47), growth on ad libitum feeding (Deuel et al., '47), ability to withstand a prolonged period of undernutrition, growth during recovery from such a period, rate of sexual development in females, fertility, and lactation performance, are all greater on diets containing liberal amounts of fat (10 to 40%) than on diets containing little or no fat (0 to 5%). The optimum fat level for growth appears to lie between 20 and 40% (Deuel et al., '47), but there is some evidence that work capacity (Scheer et al., '47) and fertility are higher on diets containing 40% fat. It is interesting to compare the data on reproduction with those presented by Deuel et al. ('47) for litter mates of the animals of the present experiment, fed ad libitum. Particularly noteworthy is the difference in fertility on the fat-free diet; the animals on ad libitum feeding had no offspring while 75% of matings in the present experiment were successful. The litters produced by the females which had recovered from the period of restricted feeding were not smaller, and in some cases were larger than those produced by females fed ad libitum.

SUMMARY

During a period of severely restricted feedings, weanling rats receiving isocaloric amounts of diets varying in fat content grow better on diets containing fat than on a similar diet lacking fat. Mortality, from uncertain causes, was highest on the diet with 5% fat. During a recovery period of ad libitum feeding, growth, fertility, and lactation were better supported by diets with liberal amounts of fat than by a fat-free diet.

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THE NET PROTEIN VALUE OF FOOD YEAST

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The concept of net protein value was proposed by Mitchell and Carman ('24) to appraise, in a single figure, the value of a food as a source of dietary protein. Such a figure combines the biological value of the protein, its coefficient of digestibility, and also its original content of "conventional" or, so-called, crude protein — i.e., $N \times 6.25$.

Very few investigations are found in the literature dealing with the determination of the coefficient of digestibility and the biological value of yeast protein using the nitrogen metabolism technic proposed by Thomas ('09) and later developed and adapted to growing rats by Mitchell ('24a, '24b, '24c) and Mitchell and Carman ('26). This biological assay procedure is considered to give the most satisfactory picture of protein utilization by the body. Mitchell ('24b) fed an undescribed type of yeast at a level of 5% protein in the diet and recorded a coefficient of digestibility of 77 and a biological value of 85.5. Still and Koch ('28) assayed a sample of dried bakers' yeast and obtained a coefficient of digestibility of 72.06 and a biological value of 25.2. Their diet contained 38% yeast, which was equivalent to about 20% crude protein. No doubt the low biological value obtained for bakers' yeast by Still and Koch was due, at least in part, to the high level of yeast protein in the diet. As no information was given in either of these studies, with respect to the protein ($N \times 6.25$) content of the yeast samples investigated, it is impossible to

use these data to compute the respective net protein values. Recently, Hughes and Hauge ('45) determined the true digestible coefficient and biological value of dried brewers' yeast and reported values of 83 and 61, respectively. Their diet contained 10% protein (N × 6.25). The yeast used by them contained about 48.7% protein. The net protein value for brewers' yeast, according to these figures, is 24.7.

Since food yeast might become an important item in the diet of tropical countries the determination of the net protein value of this yeast appeared desirable. To obtain this value, the biological values and the true coefficient of digestibility of the different yeasts were investigated by the method of Mitchell and Carman ('26). Two samples of food yeast were used. One sample was obtained from the Microbiological Section of the Chemical Research Laboratories at Teddington, England (*Torula utilis* no. 1084),[1] and the other was produced at the pilot plant of the Puerto Rico Industrial Development Co. at Río Piedras, Puerto Rico (*Torula utilis* no. 3).[2, 3] In the case of the latter, Puerto Rican sugar cane molasses was used in the growing mash. For purposes of comparison, the net protein value of a sample of dried brewers' yeast[4] was also determined.

EXPERIMENTAL PROCEDURE

In general, the technic recommended by Mitchell and Carman ('26) has been followed. Ten male Wistar albino rats of about 50–60 gm body weight were used in each trial. Metabolism cages of the type described by Still and Koch ('28) were used. This type of cage permitted the accurate collection of urine and feces samples separately. The tests were of 10 days' duration divided into 2 periods, a 3-day preliminary period, during which no samples were collected, and a 7-day experimental period during which urine and feces

[1] Supplied through the courtesy of Dr. A. C. Thaysen.

[2] Supplied through the courtesy of Mr. Rafael Fernández-García.

[3] Strain no. 3 obtained from the stock cultures of the University of Wisconsin.

[4] Fleischmann type 2019.

samples were collected daily. Ferric oxide was used as a feces marker for the accurate separation of these 2 periods. Body weights were taken at the beginning and at the end of each balance period. The food offered and residue left were accurately weighed in an analytical balance to determine the food consumed during the 7-day experimental period. The feces were collected daily and dried in an oven to constant weight at 100°C. The entire dried sample for the 7-day period was digested and aliquot samples taken for total nitrogen determination. For the accurate collection of all the voided urine, the cages were thoroughly washed daily with a hot 0.5% sulfuric acid solution. The urine plus the washings were filtered into a 2-liter bottle and kept in the ice-box with xylol as preservative. At the end of the 7-day period, these samples were well mixed and diluted to volume. Aliquot portions were taken for total nitrogen determinations. All nitrogen determinations were made according to the Kjeldahl-Gunning-Arnold method in the Official and Tentative Methods of Analysis ('35).

The 3 yeast diets were made to contain about 8% protein. In the initial and final standardizing periods, to determine the excretion of body nitrogen in feces and in urine, a dried, fat-free whole egg diet of about 4% protein was used. The experimental rations had the composition shown in table 1.

To prevent the scattering of the diet from the food cups, enough cold, distilled water was added to the amount of ration previously weighed to obtain a soft paste. With very few exceptions, this procedure completely prevented the scattering and loss of food.

RESULTS

The results of the initial and final standardizing periods are given in table 2. The purpose of these periods was to determine the excretion of the so-called "metabolic nitrogen" in the feces and the endogenous nitrogen in the urine, the former being dependent upon the dry matter consumed and the latter, upon the body weight of the rat. These factors differ some-

what in the 2 periods for each rat and in using them for the calculation of the biological values for the intermediate experimental periods, they are assumed to change in a linear fashion from the initial to the final value determination. The

TABLE 1

Composition of diets.

CONSTITUENTS:[1]	STANDARDIZING DIET	DRIED FOOD YEAST *T. utilis* No. 1084	DRIED FOOD YEAST *T. utilis* No. 3	DRIED BREWERS' YEAST
	%	%	%	%
Dried, ether-extracted whole egg	6.15			
Dried yeast		16.25	15.78	15.74
Modified Osborne and Mendel salts	3.00	3.00	3.00	3.00
Sucrose	10.00	10.00	10.00	10.00
Sodium chloride	1.00	1.00	1.00	1.00
Vegetable oil (Mazola)	9.00	9.00	9.00	9.00
Cod liver oil	1.00	1.00	1.00	1.00
Corn starch	66.07	56.75	57.22	57.26
Cellu-flour	3.00	3.00	3.00	3.00
Protein content	4.31	8.16	8.19	8.06

[1] Synthetic vitamins added as supplement to each kilo of diet: thiamine, 2 mg; pyridoxine, 2 mg; riboflavin, 8 mg.

TABLE 2

Initial and final standardizing periods. Daily excretion of metabolic and endogenous nitrogen.

	BODY WEIGHT Initial	BODY WEIGHT Final	FOOD INTAKE	FECAL NITROGEN	METABOLIC NITROGEN IN FECES PER GM OF FOOD	URINARY NITROGEN	ENDOGENOUS NITROGEN IN URINE PER 100 GM WEIGHT
	gm	*gm*	*gm*	*mg*	*mg*	*mg*	*mg*
Initial period: dried egg protein ration containing 0.74% N.							
Average of 10 rats	62.1	69.8	5.9	9.2	1.6	16.6	25.3
Standard deviation	3.3	4.0	0.4	0.5	0.2	2.3	5.5
Final period: dried egg protein ration containing 0.69% N.							
Average of 10 rats	89.4	97.9	6.2	9.7	1.5	15.4	16.5
Standard deviation	7.6	6.4	0.7	0.4	0.2	2.2	2.1

average excretion of metabolic nitrogen in the feces was 1.6 mg and 1.5 mg per gm of food consumed in the 2 periods. The endogenous nitrogen output in the urine per 100 gm body weight averaged 25.3 mg in the first standardizing period and 16.5 mg, in the second.

TABLE 3

Nitrogen metabolism of rats on yeast rations.
(Results expressed on the daily basis.)

	BODY WEIGHT		FOOD INTAKE	NITROGEN		
	Initial	Final		Intake	In feces	In urine
	gm	*gm*	*gm*	*mg*	*mg*	*mg*
	Dried food yeast *(T. utilis)* No. 1084 ration					
Average of 10 animals	68.9	68.9	4.32	61.6	13.8	42.7
Standard deviation	5.7	4.5	0.69	10.0	2.2	7.1
	Dried food yeast *(T. utilis)* No. 3 ration					
Average of 10 animals	70.2	69.0	4.07	58.1	13.6	43.0
Standard deviation	3.8	5.4	0.68	10.1	3.1	5.2
	Dried brewers' yeast ration					
Average of 10 animals	75.1	82.9	5.71	81.5	20.6	36.0
Standard deviation	6.8	6.3	0.60	12.6	2.5	6.0

All of the nitrogen metabolism data collected in the different trials are assembled in table 3. The net protein values, digestion coefficients and biological values computed from these data are summarized in table 4. The method of calculating the values has been fully explained by Mitchell ('24a, b) and by Mitchell and Carman ('26).

TABLE 4

Coefficient of true digestibility, biological value and net protein value of the nitrogenous substances present in yeast.

	DRIED FOOD YEAST *T. utilis*		DRIED BREWERS' YEAST
	No. 1084	No. 3	
(a) % Protein (N × 6.25) in the yeast samples	49.25	50.69	50.81
(b) Total fecal nitrogen [1]	13.8	13.6	20.6
(c) "Metabolic nitrogen" in feces [1]	6.6	6.2	8.8
(d) Food nitrogen in feces (b–c) [1]	7.2	7.4	11.8
(e) Total nitrogen intake [1]	61.6	58.1	81.5
(f) Nitrogen absorbed (e–d) [1]	54.4	50.7	69.7
(g) Total urinary nitrogen [1]	42.7	43.0	36.0
(h) Endogenous nitrogen in urine [1]	14.9	15.3	14.6
(i) Food nitrogen in urine (g–h) [1]	27.8	27.7	21.4
(j) Food nitrogen retained (f–i) [1]	26.6	23.0	48.3
(k) Biological value: $\frac{j}{f} \times 100$	48.8	45.3	69.3
(l) True digestibility coefficient: $\frac{f}{e} \times 100$	88.3	87.3	85.5
(m) Net protein value (a × k × l)	21.2	20.0	30.1

[1] Results expressed in milligrams of nitrogen per day. Values are averages of 10 animals.

DISCUSSION

The true coefficients of digestibility of the 3 yeasts investigated were computed by assessing the metabolic nitrogen in the feces. The true digestibilities thus obtained were as follows: 85.5 for brewers' yeast, 88.3 for *Torula* yeast no. 1084, and 87.3 for *Torula* yeast no. 3.

The nitrogen metabolism data indicate a much better utilization of the absorbed nitrogen derived from the brewers' yeast than of that derived from either one of the *Torula* samples. The average biological values computed from these data were 69.3 for brewers' yeast and 48.8 and 45.3, respectively, for the *Torula* samples no. 1084 and no. 3. This dif-

ference in biological value of the brewers' and *Torula* yeast is noteworthy. It might be ascribed to several variable factors resulting from the difference in species, the nutrients used in growing these organisms and the methods of manufacturing. Some of these factors are the content of protein and non-protein nitrogen in the yeasts, the amino acid make-up of their individual proteins, the availability of these amino acids, etc. In regard to this last factor, it is well known that in the case of soy bean, heat treatment improves the nutritive value of the raw product by increasing the availability of cystine (Mitchell et al., '45). Recently, Axtmayer ('46) has claimed that autoclaving improves the nutritive value of yeast. It has been known for some time that, although yeast protein contains about 2% methionine, apparently, this is not in a readily available form. Perhaps the processing to which a specific yeast is subjected during the recovery and drying operations in the factory may modify its final nutritive value.

In considering the biological value of yeast protein (N × 6.25), it should always be remembered that an appreciable amount of the yeast nitrogen (about 12 to 20%) is in the form of non-protein nitrogen. This should not constitute an argument against evaluating yeast as a good source of protein because of its high net protein value. We have obtained a net protein value of 30.1 for brewers' yeast, 21.2 for *Torula* yeast no. 1084 and 20.0 for *Torula* yeast no. 3. Even in the case of the food yeasts, with biological values below that of brewers' yeast, the net protein values were the same or above those of other foods such as raw soy flour 21.4% (Mitchell et al., '45), and rolled oats, navy beans, white flour and whole corn, which were 10.6, 6.0, 5.0 and 3.5%, respectively (Mitchell, '27). This proves that food yeast ranks high as a source of dietary protein.

SUMMARY

Using the nitrogen balance method, the protein values of 2 samples of food yeast *(Torula utilis)* and one of brewers' yeast were determined. The *Torula* yeasts, no. 1084 and no. 3, and the brewers' yeast were found, respectively, to be 88.3,

87.3 and 85.5% digestible, to possess biological values of 48.8, 45.3 and 69.3 and to have net protein values of 21.2, 20.0 and 30.1.

The 2 *Torula* yeasts, the one from England, no. 1084, and the one from Puerto Rico, no. 3, have practically equal nutritive coefficients. The superior net protein value of the brewers' yeast over that of the *Torula* samples is due exclusively to its higher biological value since the protein ($N \times 6.25$) content and digestible coefficients of the 3 yeast samples are practically the same.

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